EFFECT OF ENZYME SUPPLEMENTATION ON THE NUTRITIVE VALUE OF CANOLA MEAL FOR BROILER CHICKENS

A thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Dongsheng Liang

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Effect of Enzyme Supplementation on the

Nutritive Value of Canola Meal for Broiler Chickens

BY

Dongsheng Liang

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University

of Manitoba in partial fulfillment of the requirements of the degree

of

Master of Science

ABSTRACT

The purpose of the research was to explore the potential for improving the nutritive value of canola meal (CM) by pretreatment with a combination of enzymes during the canola seed crushing process. A number (12) of carbohydrase-like (ie., α-galactosidase, pectinase, cellulase) and (2) phytase enzymes were evaluated *in vitro* (80% moisture content) for their ability to hydrolyze oligosaccharides and phytate and to depolymerize the non-starch polysaccharides (NSP) of canola meal. Enzymes were selected based on their ability to reduce carbohydrate and phytate, their specific enzyme activity and source (manufacturer). The selected enzymes were combined into four enzyme blends. Based on *in vitro* (80% moisture content) results, it showed that there was an additive effect for enzyme blends on NSP (one blend) and phytate (three blends) reduction compared to single enzymes. However, the enzyme pretreated canola meals (16-20% moisture content) did not show any NSP and phytate reduction. When enzyme pretreated canola meals were fed (30% of diet) to 4-day old broiler chickens for a 2-week period improvements in growth performance (one blend) relative to a control diet were noted ($P<0.05$). Compared to the control diet, enzyme pretreated canola meals significantly increased body weight gain and improved feed to gain ratio (one blend). Three enzyme pretreated canola meal based diets resulted in improved ileal protein digestibility ($P<0.05$) and phytate digestibility ($P<0.05$), but only two enzyme improved ($P<0.05$) apparent metabolizable energy (nitrogen corrected) value. The study indicated that there is potential for improvement of the quality of canola meal by enzyme (blend) pretreatment. However, the relatively low moisture content in desolventized canola meal meal would appear to limit the effectiveness of pretreatment of canola meal during the

crushing process and further research is needed to realize optimum effects of added enzymes.

Studies were also conducted to investigate how and why enzyme supplementation influenced feed intake. To explain the mode of action of protease enzymes, casein or hydrolyzed casein and monosaccharides based diets with or without protease supplementation were used. The results showed that both the protease supplemented diet and the hydrolyzed casein diet reduced feed intake significantly ($P<0.05$), but only the hydrolyzed casein diet increased jejunal protein digestibility and free amino acids in plasma. Based on an *in vitro* study (mimicking the crop environment), it may be suggested that a high level free amino acids could exist in the crop. Therefore, the mode of feed intake reduction could be caused by high level of free amino acid in the crop that would stimulate the osmoreceptor. Increased jejunal amino acids digestibility and the level of plasma free amino acids may further reduce feed intake with the hydrolyzed casein diets.

ACKNOWLEDGMENTS

I would like to express my sincere thanks to my adviser Dr. W. Guenter, not only for his continued guidance and tireless effort and supervision of this work, but also for the opportunity he offered to me as an MSc. student. I would also like to thank Dr. B. A. Slominski for his instruction in my study and research. My sincere thanks to my committee members, Dr. S. Arntfield, Dr. G. H. Crow and particularly D. M. Veira for his encouragement to complete my studies.

I am also indebted to the administrative staff and graduate students for their friendship throughout the years. Many thanks to H. Muc and P. Mills for their technical assistance.

Thank you to NSERC operating and Canadian Biosystem for their financial contributions during the course of this work.

Last but not the least, my sincere thanks go to my wife YanFeng Wu and son Siyuan for all their love, understanding and encouragement throughout my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	amino acid
AMEn	apparent metabolizable energy(nitrogen corrected)
AME	apparent metabolizable energy
ANF	anti-nutritional factor
BW	body weight gain
CM	canola meal
DM	dry matter
FCR	feed conversion ratio
FI	feed intake
GL	glucosinolate
NSP	non-starch polysaccharide
SBM	soybean meal
TMA	trimethylamine
TME	true metabolizable energy

1. INTRODUCTION

Canola is the name for genetically altered form of rapeseed with less than two percent erucic acid and less than 30 micromoles of glucosinolate per gram of air-dried oil-free meal(Bell, 1993). The development of low erucic acid and low glucosinolate cultivars of canola has resulted in increased usage of canola meal in poultry rations(Clandinin 1989). Canola is the most important oil and protein crop in Canada, due to its high yield and high nutritive quality of both oil and protein.

The commercial canola meal usually is made from a blend of *Brassica napus* and *Brassica rapa* seed by a combination of expelling and solvent extraction, and it contains 34 to 38% crude protein and has a well balanced amino acid profile and high levels of sulfur-containing amino acids relative to soybean meal. It is rich in essential minerals and seems to be rich in choline, biotin, folic acid, niacin, riboflavin and thiamin (Bell, 1993). Its chemical composition, however, may be influenced by environmental conditions during the growing of the crops, by harvest conditions, and to a minor extent by cultivar and processing of the seed and meal.

Although canola meal contains a high concentration of crude protein, it also contains antinutritional substances that include fibre, phytate, sinapine and tannins. Even though canola has a low level of glucosinolate relative to the original rapeseed, the glucosinolate content is still considered to be at a level that influences its exclusive use in monogastric animals.

Among the antinutritional factors, the high fiber level is one of the greatest restrictions to canola meal use in poultry diets. Dietary fiber accounts for approximately one-third of the meal, and it consists of cellulose (4-6%), non-cellulosic polysaccharides (13-16%), lignin and polyphenols (5-8%), and protein and minerals associated with the fiber fraction (Slominski and Campbell, 1990). Thus, the relatively low AMEn content and the less than optimal protein and amino acid digestibilities caused by the high level of dietary fiber are considered to be the main factors that restrict canola meal use in poultry diets.

Because much of the fiber is in the hull, the negative effects of the fiber can be reduced by dehulling. Leslie et al. (1973) has demonstrated with rats that the digestibility of rapeseed protein was increased about 10-20% by dehulling. However, the moderate levels at which canola meal is used in poultry diets, combined with the modest improvement in chick performance with dehulled meal, make the economics of dehulling questionable (Shires et al., 1983). Genetic selection provides another way to improve the canola meal quality. It may be possible to breed a new variety of canola with thin seed coat, low fiber level and more protein (Newkirk et al., 1997; Slominski, 1997).

The use of feed enzymes in poultry diets is now commonplace in barley, wheat and oat-based diets in many countries (Bedford and Schulze, 1998). Enzymes have the greatest potential use in diets that contain antinutritional factors that hinder nutrient availability. The non-starch polysaccharides (NSP) in feedstuffs have been the main target of commercial feed enzymes. These NSPs which include cellulose, β-glucans, arabinoxylans, and pectins may increase viscosity of digesta resulting in decreased nutrient digestibility. Phytate another

anti-nutrient factor, is a complex of inositol and phosphorus which render the phosphorus unavailable (Ravindran and Bryden, 1997). Feed enzyme supplementation can improve the NSP and phosphorus availability, reduce the negative impact of these indigestible residues, render certain nutrients more-readily available for absorption from wheat and barley diets and improve the bird performance (Annison and Choct,1991; Sebastian et al., 1998).

There are, however, few reports on the addition of enzyme to improve canola meal quality. Nevertheless, most studies have indicated that cabohydrase or protease supplementation improve feed conversion ratio (Simbaya et al, 1996; Guenter et al., 1995; Slominski et. al., 1997), but Alloui et al. (1994) and Sosulski et al. (1990) reported that the addition of carbohydrase and protease enzyme did not significantly improve bird performance. Since the optimum pH was around 5 ~ 7 for exogenous enzyme to work efficiently, the activity of exogenous enzymes may be reduced by the low pH in the gizzard (pH 2.0). To maximise the enzyme action on the substrate, pretreatment of canola meal with enzyme was explored.

The major objective of this study was first to explore the potential for improving canola meal utilization through enzyme pretreatment with an *in vitro* system, secondly to develop the best enzyme blend to be effective in cell wall depolymerization and to apply the best enzyme blend to a practical situation through mimicking the canola meal processing condition and thirdly to investigate how this pretreated canola meal would affect chicken performance and phytate and NSP hydrolysis, ileal protein availability and energy improvement. Since there are a few studies (Simbaya et al., 1995; Sebastian et al., 1994;

Jackson, 1998) showing that enzyme supplementation reduced feed intake, the additional objectives were to demonstrate the feed intake reduction with protease enzyme feeding and to investigate the reason for the feed intake reduction.

2. LITERATURE REVIEWS

2.1 QUALITY CHARACTERISTICS OF CANOLA MEAL

2.1.1 The general composition of canola meal

The main components of canola meal include protein, carbohydrates, crude fiber, lipids and ash (Table 1). Compared to soybean meal, canola meal contains less protein and higher fiber. The amount of lipid in canola meal, 4-5%, is higher than in soybean meal, and depends on the extent of canola oil extraction and the amount of gums added back to the meal during canola processing. Canola meal is a relatively good source of essential minerals and appears to be a good source of selenium relative to soybean meal. However, the availability of most minerals in canola meal is lower than that of soybean meal (Nwokolo and Bragg, 1977), and may be due to the high dietary fibre and the potential for the high level of phytate to complex mineral. Although there is limited information published on vitamin content, canola meal seems to be rich in choline, biotin, folic acid, niacin, riboflavin and thiamin (Table 1).

Generally speaking, the composition of canola meal is reasonably consistent, because the pooling of meal during processing minimizes variation. However, variation has been documented among cultivars, affected by environment and season and among crushing plants (Bell and Keith, 1991).

Table 1. Chemical composition of canola meal and soybean meal (as fed basis)

Component	Canola meal [1]	Soybean meal[2]
Dry matter %	90	90
Crude protein %	34	48.5
Crude fibre %	12	3.9
Total dietary fibre %	33	-[3]
Ether extract %	3.8	1
Ash %	4.8	-
Mineral		
Calcium %	0.63	0.27
Phosphorus %	1.01	0.62
Non-phytate P %	0.3-0.52	0.22
Sodium %	0.7	-
Chlorine %	0.1	0.05
Copper mg/kg	5.7	15
Iron mg/kg	142	170
Manganese mg/kg	49.2	43
Molybdenum mg/kg	1.4	-
Zinc mg/kg	69	55
Selenium mg/kg	1.1	0.1
Vitamin		
Choline %	0.67	0.27
Biotin mg/kg	1.1	0.32
Folic acid mg/kg	2.3	1.3
Niacin mg/kg	160	22
Pantothenic acid mg/kg	9.5	15
Riboflacin mg/kg	5.8	2.9
Thiamin mg/kg	5.2	3.2
Pyridoxine mg/kg	7.2	5
Vitamin E mg/kg	14	3

1 Canola Council of Canada (1997).
2 National Research Council (1994).
3 Data not available.

2.1.2 Amino acids profile and digestibility

The average protein content of canola meal is 34-38%. As a protein supplement in animal diets, canola meal has a well-balanced amino acid profile relative to other protein supplements such as soybean meal (Table 2). Canola meal has a high content of methionine as a percent of total protein (2% as compared with 1.5% in soybean meal). In contrast, however, the lysine content of canola is lower than soy (Dale, 1996). Thus, canola meal has an amino acid distribution very complementary to soybean meal. For this reason, the two meals are often included in the same ration and feeding trials show animals perform better when fed the mixture than when fed either meal alone (Canola Council of Canada, 1997).

The digestibility of amino acids is one of the important factors for estimating the nutritive value of protein feedstuffs for poultry. Most of the experiments (May and Bell, 1971; Sauer et al., 1982 Table 3) found that the digestibility of canola/rapeseed proteins and the true amino acid availability in monogastric animals was about 10% lower than that of soya proteins.

Many explanations for the low digestibility of canola meal compared to soybean meal have been proposed, and include high fiber content, and the presence of tannins, lignins, pectins (de Lange et al., 1990) and glucosinolates. Fibre may be the most important factor to influence the protein digestibility. Fiber can induce a faster passage rate, which reduces the opportunity for digestion, and increases endogenous nitrogen loss through abrasive action or binding endogenous protein (Sauer and Thacker, 1986; Imbeah and Sauer, 1991).

Table 2. Amino acid content of canola meal and soybean meal (% as fed basis)

Amino acids	Canola meal			Soybean meal
Protein	34.00[1]	34.80[2]	37.67[3]	50.00[4]
Lysine	1.91	1.94	2.24	3.07
Methionine	0.71	0.71	0.77	0.68
Cystine	0.85	0.87	1.08	0.71
Threonine	1.53	1.53	1.71	1.94
Arginine	2.04	2.08	2.34	3.66
Glycine	-[5]	1.82	1.96	2.07
Serine	-	1.53	1.76	2.54
Histidine	1.22	0.93	1.39	1.31
Isoleucine	1.32	1.37	1.6	2.45
Leucine	2.48	2.47	2.74	3.83
Phenylalanine	1.35	1.44	1.54	2.52
Tyrosine	1.01	1.09	1.14	1.82
Valine	1.79	1.76	2.06	2.5

[1] Canola Council of Canada (10% moisture content, 1997).
[2] National Research Council (12% moisture content, 1994).
[3] Bell and Keith (10% moisture content, 1991).
[4] Nutrition Guide, Rhone-Poulenc Animal nutrition (10% moisture content, 1989).
[5] Data not available.

Table 3. Protein and amino acids availability[1] of canola meal and soybean meal in poultry (% of total in the meal)

	Canola meal					Soybean meal
	TAAA				AAAA	TAAA
Lysine	83.3[2]	78[3]	82.8[4]	80[5]	74.8[4]	92.2[6]
Methionine	91.3	88	82.8	90	79.6	94.4
Cystine	81.7	73	-[7]	75	-	91.7
Threonine	87.0	78	79.0	78	71.1	92.1
Arginine	90.3	89	87.5	90	78.0	96.0
Glycine	-	-	-	-	-	94.9
Serine	84.7	-	80.8	-	72.1	94.5
Histidine	89.3	86	92.6	85	87.4	94.0
Isoleucine	89.7	83	82.4	83	76.4	93.0
Leucine	91.7	86	86.3	87	81.0	92.0
Phenylalanine	91.3	86	86.0	87	80.8	94.6
Tyrosine	88.0	-	82.4	-	74.4	94.1
Valine	89.3	81	80.7	78	74.7	92
Average	**88.1**	**82.8**	**83.9**	**83.3**	**77.3**	**93.5**

[1] TAAA, true amino acid availability; AAAA, apparent amino acid availability
[2] Salmon (1984b).
[3] Canola Council of Canada (1997).
[4] Lee et al. (1995).
[5] National Research Council (1994).
[6] Nutrition Guide, Rhone Poulenc Animal Nutrition (1989).
[7] Data not available.

Finlayson (1974) has demonstrated that approximately 7% of the seed nitrogen is tightly bound in the hull and is of a form not readily digested. Furthermore, Lessire et al. (1991) reported that the digestibility of protein in hull is zero resulting in low amino acids digestibility for canola meal.

It has also been demonstrated that the processing conditions have significant effects on protein and amino acid availability. During processing, the canola meal is heated three different times, i.e., during the conditioning, expelling, and desolventizing steps. It is well known that excess heating may damage protein and render amino acids, particularly lysine unavailable to animals (Hancock et al., 1990). Anderson-Hafermann et al. (1993) reported that the large effect of overheating on lysine digestibility was due to the lysine binding in a Maillard reaction thus preventing liberation during digestion.

2.1.3 Metabolizable energy

Available energy is expressed in several ways and is affected by variability among samples, by processing of the seed, by species and age of animal being fed the meal and by the method used to assess energy content. Apparent metabolizable energy (AME) and true metabolizable energy (TME) have been widely used as an index of the available energy in feeds for poultry. AME is generally used for chicks, and TME is usually determined with adult roosters.

The gross energy of canola meal is around 4450±48 kcal/kg dry matter (Bell and Keith, 1991), and was nearly the same as soybean meal. However, the AMEn is about 1900kcal/kg for growing birds and 2000kcal/kg for adult birds (Clandinin and Robblee, 1983, Table 4),

Table 4. Available energy value for canola meal (kcal/kg)

AMEn	TME	TMEn	Reference
1980		2090	Lee et al. (1995).
2000		1955	Canola Council of Canada (1997).
	2493		Salmon et al. (1984a)
	2049	1964	Barbour and Sim (1991)
	2400		Salmon et al. (1984b)

which is about 440kcal/kg and 415kcal/kg lower than soybean meal, respectively. This can be explained by its higher fiber content for canola meal compared to soybean meal (crude fiber 11% vs 3%) and a lower level of available carbohydrate in canola meal than for soybean meal (Lodhi et al., 1969).

Low metabolizable energy is the major limitation for canola meal use in high energy poultry diets. It can be concluded that improving its energy value and protein availability can further increase the usefulness of canola meal for poultry.

2.2 ANTINUTRITIONAL FACTORS OF CANOLA MEAL

Canola meal is a good source of high-quality protein and is in widespread use as a protein source in feed formulation for poultry and other animals (Bell, 1993). However, the nutritional value of canola meal is influenced by its contents of different antinutritional factors such as fiber, glucosinolate, aromatic choline esters and phytate (Table 5) of which the fiber and glucosinolates are by far the most important (Bjergegaard et al., 1998; Jensen, et al., 1995). The high fiber content of canola meal is now recognized as the primary factor that resulted in the relatively low metabolizable energy and digestibility of protein of canola meal(Bell, 1993).

Table 5. Typical antinutritional composition of canola meal[1]

Antinutritional components	Average
Crude fibre %	12
Acid detergent fibre %	17.2
Neutral detergent fibre %	21.2
Cellulose %	4.6
Non-starch polysaccharides %	16.1
Soluble NSP %	1.4
Insoluble NSP %	14.7
Oligosaccharides %	2.3
Tannins %	1.5 - 3.0
Sinapine %	0.6 - 1.8
Phytic acid %	3.0 - 6.0
Glucosinolates (μmol g^{-1})	18.3
Aliphatic	14.1
Progoitrin[2]	6.4
Gluconapin	3.1
Glucobrassicanapin	0.4
Gluconapoleiferin	0.7
Indole	4.2

[1] Modified from Canola Council of Canada (1997)
[2] Simbaya (1996)

2.2.1 Dietary Fiber and Oligosaccharides

The dietary fiber of canola meal accounts for approximately one-third of the meal. It results in a low energy yield, protein utilization and amino acid availability, and has been recognized as one of the main anti-nutritional factors contributing to the low energy limiting the use of canola meal in poultry diets.

The high fiber content of canola meal is due largely to the high proportion of hull in relation to the size of the seed, representing about 16% of the seed weight and about 30% of meal weight. Hull contains a high level of NSP and lignin, which may reduce the protein digestibility and energy level. Jensen et al. (1995) found a strong relationship between hull content and lignin content ($P<0.01$) and total dietary fiber ($P<0.05$), and significant negative correlations between hull content and true protein digestibility ($P<0.01$) and energy digestibility ($P<0.05$). He suggested that the reason was the indigestible protein fraction in the meal which may have resulted from binding to, or being encapsulated by fibrous components of the meal.

However, a large amount of NSP in canola meal not only comes from the hull but also is contributed by the endosperm. Bjergegaard et al. (1991) provide envidence that dietary fiber fractions isolated from the germ had a more negative effect on the digestibility of both protein and energy than dietary fiber isolated form the hulls. Jensen et al. (1995) also demonstrated that the triple low (low erucic, low glucosinolate and low fiber) samples had low hull contents. However, the triple low cultivars do not have a higher digestibility of nutrients than double low (low erucic and low glucosinolate) cultivars because they have

high dietary fiber in the germ. The reason is that the endosperm cell wall of canola meal can serve as a physical barrier between digestive enzymes and nutrients contained within the cells, and can either prevent access entirely or delay digestion of the nutrients to a more posterior portion of the gut (Campbell and van der Poel, 1998).

The dietary fiber components of canola meal, which include lignin polyphenols, non-starch polysaccharide (NSP), cell wall protein and associated minerals (Simbaya, 1996; Table 6), are not digested in the small intestine of poultry but are degraded to some degree by the microflora of the lower intestine. A balance study with laying hens showed that the digestibility of the NSP fraction of canola meal was below 3% (Slominski and Campbell, 1990). The low digestibility (3%) of fiber components was the main factor responsible for depressing protein digestibility, amino acids availability and the ME value in the meal. Newkirk et al. (1997) reported that total dietary fiber was negatively related to AMEn (kcal kg^{-1}= −183 TDF + 7179.9 $P<0.02$ R^2=0.78) and ileal protein digestibility (%= −1.567 TDF + 121.6 $P<0.3$ R^2=0.73).

The oligosaccharides, raffinose and stachyose, also have been considered antinutritional factors affecting utilization of energy from soybean meal in poultry (Coon et al., 1990). They are not digested by endogenous enzymes and pass intact through the intestine of monogastric animals, and are extensively fermented in the hindgut. Opinions vary in the literature regarding the potential antinutritional effects of oligosaccharides. By using oligosaccharide free canola meal compared with normal canola meal, Slominski et al. (1994) indicated that oligosaccharides do not constitute a major antinutritive effect in canola meal. Their results were contrary to Coon et al. (1990) who indicated a depressing

Table 6. Dietary fiber components of canola meal

Component	%
Cellulose	4-6
Non-cellulosic polysaccharide	13-16
Lignin and polyphenols	8
Cell wall protein	3.50
Cell wall minerals	1
Total	28.0-34.5

Simbaya (1996)

effect of oligosaccharides on utilization of energy from soybean meal. The difference observed between the two studies was that canola meal contained more ethanol-soluble material but less of the oligosaccharides than soybean meal (Slominski et al., 1994).

However, excessive fermentation in the small intestine may interfere with the normal physiological process of nutrient digestion. Misir and Marquardt (1978) reported that adding antibiotics to poultry diets containing highly soluble NSP markedly improved bird performance. The authors suggested that the antibiotics might inhibit or suppress the intestinal microflora that compete with the host for available dietary nutrients.

2.2.2 Glucosinolate (GL) problem

With the development of the low GL varieties of canola, a significant breakthrough was achieved in meal quality. Modern cultivars have reduced glucosinolate to 10 to 12% of the original levels (110 - 150 μmols g^{-1}), resulting in cultivars with levels around 10 ~12 μmols g^{-1}. However, in many cases, the presence of glucosinolates in canola meal is still considered as a factor restricting its inclusion level in the diet for monogastric animals, owing to both the physiological and anti-nutritional impact (Bell, 1993)

There are two main groups of glucosinolates, the aliphatic and the indole, each of which contains several unique glucosinolates present in the seed, with the general structure of β-D-thioglucopyranosides. The most common aliphatic glucosinolates in canola meal are progoitrin, gluconapin, gluconapoleiferin and glucobrassicanapin, with progoitrin being responsible for the major antinutritive effect in canola meal (Fenwick and Curtis, 1980).

These intact glucosinolates are relatively innocuous to poultry (Sarwar et al., 1981). Hydrolyzed products of the glucosinolates, particularly isothiocyanates, thiocyanates, nitriles and oxazolidinethiones, are considered to be more harmful than the intact glucosinolate (Tookey et al., 1980). Isothiocyanates are responsible for the pungent odor and the problem of palatability encountered when animals receive high levels of high-glucosinolate rapeseed meal (Fenwick and Curtis, 1980). Isothiocyanates are known to depress iodine metabolism in the thyroid gland, and this may have goitrogenic or other metabolic effects (Vermorel et al., 1988). Thiocyanates that may be formed from the hydrolysis of indolyl glucosinolate also affect uptake of iodine by the thyroid gland, but this effect can be reversed by supplemental dietary iodide (Fenwick and Curtis, 1980). Oxazolidinethiones formed from the hydrolysis of aliphatic glucosinolates are primarily responsible for the goitrogenic effect of canola meal because they irreversibly block uptake of iodine by the thyroid gland. Nitriles have high toxicity and may affect the liver and kidney to a great extent (Campbell and Schöne, 1998). Generally speaking, glucosinolates or their metabolic derivatives decrease the growth rates of broilers, increase the thyroids and liver sizes and cause hemorrhagic liver syndrome (Fenwick and Curtis, 1980).

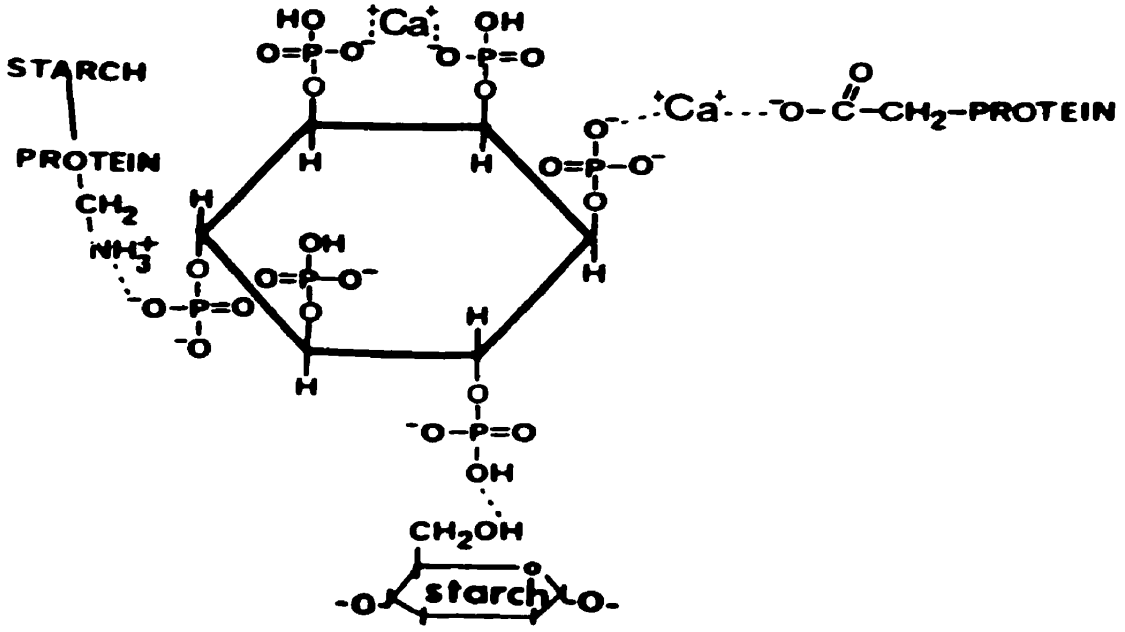
Although glucosinolates may have negative effects on utilization of energy or other nutrients, the effect of glucosinolates at current levels in Canadian canola meal ($<30 \mu molg^{-1}$) is considered less than that of fiber (Bell and Keith, 1991). Also reduction of the glucosinolate levels to <5 $\mu mol\ g^{-1}$ resulted in only marginal improvement in nutrient digestibility or pig performance (Bell et al., 1991). However, when very low glucosinolate canola meal was tested with broiler chickens, performance was better then with commercial

canola meal and similar to soybean meal (Classen et al., 1991). Jensen (1999) also reported that the feeding value of rapeseed meal with a glucosinolate content < 10 μmol g^{-1} seed was higher than rapeseed varieties with around 20 μmol g^{-1}. These researchers indicated that genetic reduction in glucosinolate content in canola seed is both possible and desirable.

2.2.3 Phytate

Phytate {myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)} is found in all plants, and is regarded as the primary storage form of P and inositol in almost all seeds (Ravindran et al., 1995). The phosphorus contained in the phytate molecule is unavailable to the monogastric animal. In addition, phytate has a strong capacity to form complexes with divalent and trivalent cations, such as calcium, magnesium, zinc, iron, manganese and copper (Figure 1). These complexes are insoluble at physiological pH and render these elements unavailable for intestinal absorption. Nwokolo and Bragg (1977) found that phytate had a significant inverse relationship with the availability of P, Ca, Mg and Zn in growing chicks. Such interference may lead to increased dietary mineral requirements for animals. In poultry nutrition, some attention has been given to the effect of phytate in increasing the dietary requirements of calcium. Nelson et al. (1968) found that the calcium level was increased from 0.5% to 0.95% corresponding with a no phytate purified diet and a practical diet containing 0.95% phytic acid. O'Dell et al. (1964) reported that the addition of phytic acid markedly decreased the biological availability of zinc in broiler chicks. The authors also reported that excess calcium in the presence of phytate aggravated the zinc deficiency symptoms.

Figure 1. Possible interactions of phytic acid with minerals, protein, and starch



Thompson (1986)

Phytate in plant feedstuffs has the potential to bind proteins and negatively affect protein digestibility (Carnovale et al., 1988). It was believed that the anionic phosphate groups of phytate posses the ability to bind with cationic groups of protein, in particular the amino groups of basic amino acids (ie., lysine, arginine and histidine). *In vitro* studies have shown that phytate-protein complexes are insoluble and less subject to attack by proteolytic enzymes than the same protein alone, and this interaction may influence the digestibility of protein (Ravindran et al., 1995).

Furthermore, phytate is also able to bind endogenous proteins such as trypsin and chymotrypsin in the gastrointestinal tract (Singh and Krikorian, 1982), and these enzymes are released into the gut from the pancreas and, if they become bound to the phytate molecule, protein and amino acid digestibility could be reduced.

Canola meal contains about 1.22% total P of which 0.7-0.8% is phytate-bound, which indicates that canola meal has about twice as much phytate P compared to soybean meal (SBM). High phytate level could depress protein and mineral availability through a phytate-mineral-protein complex. In addition, unavailable or minimally available phytate P, when high levels of canola meal are fed to the monogastric animal, may contribute to phosphorous pollution via animal waste (Campbell and Schöne, 1998).

2.2.4 Other anti-nutritional factors that affect the nutritional value of canola meal

Other anti-nutritional compounds, which influence canola meal quality, are phenolic compounds that include sinapine and tannins. These two factors are not as important as fiber and glucosinolates, but comprise around 3-5% of the meal, and may influence the nutritional value of canola meal. It was indicated that the phenolic compounds may contribute to the

dark color, bitter taste and astrigency of canola meal and may affect feed intake (Campbell and van der Poel, 1998).

Sinapine is well known for its production of an off-flavor or "fishy egg" by susceptible hens (Rhode Island Red), since this breed of laying hens lack the trimethylamine(TMA) oxidase enzyme and cannot handle the high yield of choline hydrolyzed from the sinapine in the gut. The trimethylamine therefore is deposited in the egg rather than being excreted as the water soluble and odorless trimethylamine oxide. Broilers do not accumulate TMA in their tissues since their TMA oxidase levels are high (Butler et al., 1982).

It is believed that egg taint occurs when sinapine levels exceed 1g kg^{-1} diet. Removal from or reduction of sinapine in canola via breeding is a possible area for improvement of canola meal since its competitor SBM contains no sinapines (Blair and Reichert, 1984).

Tannins are high molecular complex phenolic compounds. Compared with the other anti-nutritional factors, tannins are present in canola meal at lower level, about 1.5-3.0 % (Bell, 1993). Tannins may form soluble or insoluble complexes with proteins through multiple hydrogen bonds or covalent links, thus they interfere with nutrient utilization of canola meal. More important is the fact that tannins are known to interfere with digestive enzymes, thereby reducing protein utilization in monogastric animals. However, Mitaru et al. (1982) reported that, in contrast to sorghum tannins that inhibited α-amylase activity by 79%, the canola tannins had no effect on α-amylase activity. Broiler feeding trials indicated that inclusion of tannin-containing canola meal hulls did not affect performance differently then inclusion of tannin-free soybean hulls (Mitaru et al., 1983).

Generally, canola meal is limited in use as poultry feed because of the presence of antinutrional factors, which include high fibre, glucosinolate, phytate, tannins and sinapine. These antinutritional factors incurred low metabolizable energy and low amino acid availability. It is apparent that canola meal could be more competitive in the feed market if it had more metabolizable energy, more protein and higher amino acids availability. It is known that the nutritional quality of canola meal can be improved by biotechnology, physical processing or chemical supplementation.

2.3 IMPROVING THE NUTRITIVE VALUE OF CANOLA MEAL

2.3.1 Dehulling of canola seed and breeding for low fiber cultivars

Various attempts have been made to improve the nutritive value of canola meal by reducing its fibre content. Dehulling is the most obvious means of removing fibre. The high fiber content of canola is largely due to the high proportion of hull in relation to the size of the seed. It is possible that a reduction in the fiber content of canola by removal of the hulls during processing may increase the protein level, and improve digestibility of the protein. Leslie et at. (1973) demonstrated with rats that the digestibility of canola protein was increased by dehulling. However the moderate levels at which canola meal is used in poultry diets, combined with the modest improvement in chick performance ($P<0.05$) make the economics of dehulling questionable (Shires et al., 1983).

The negative effects of the high fibre content can be reduced or eliminated by plant breeding, proper processing or a combination of breeding and processing (Jensen, et

al.,1995; Liu, et al.,1995). It is possible to breed low-fiber and high protein cultivars. Selection for yellow seed coat colour has been a priority in plant breeding in an attempt to reduce the fibre content of the meal. Thinner hulls were considered to be responsible for the lower fiber content in yellow seeded canola (Slominski, 1997). Simbaya et al. (1995) have demonstrated that yellow seeded canola has more protein and sucrose and lower dietary fiber than brown seeded canola meal. Compared with brown seeded canola meal, yellow seeded canola meal has higher energy and available amino acids.

2.3.2 Exogenous enzyme supplementation

The application of industrially produced enzymes, amylase and protease, to enhance starch and protein utilization in animal nutrition dates back to the late 1950's or early 1960's (Jensen et al., 1957; Burnett, 1962). The widespread use of barley and wheat as raw materials for poultry feed triggered the use of enzymes to solve problems such as sticky droppings and poor animal performance in terms of growth and feed conversion. These kinds of feeds are rich in nutrients but also in ANF. Supplementation of exogenous enzyme to those diets can eliminate ANF, enhance feed digestibility, improve feed conversion ratio, reducing feed cost. (Bedford and Morgan, 1996).

a) A resume of exogenous enzyme use in poultry diets.

Biologically, enzymes are functional proteins, catalyzing all metabolic processes in animals, plants and microorganisms. Every enzyme has its own unique properties, like

specific activity, substrate affinity, stability, pH and temperature sensitivity, and can be classified by the substrate upon which it reacts (Table 7).

Enzymes used as feed supplements today in poultry nutrition are produced from fungi or bacteria. Based on their substrate, enzymes can be categorized as carbohydrases, proteases and phytases (Puchal and Mascarell, 1999; Ferket, 1993). Carbohydrases and phytase are the most common enzymes utilized in poultry diets.

b) Using enzyme with barley and wheat based diets

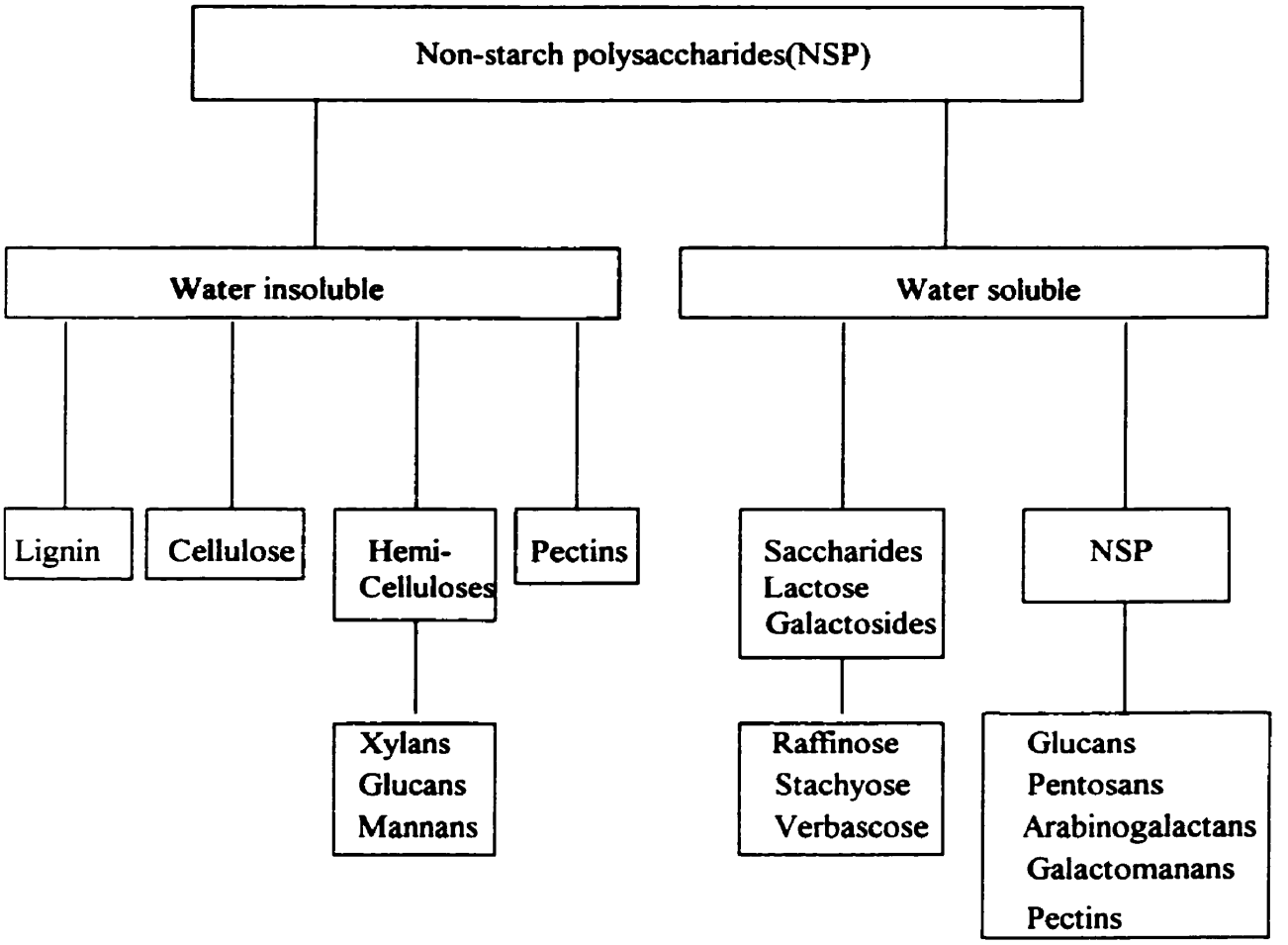
Cereal-based diets contain a class of poorly digested substances called non-starch polysaccharides (Figure 2), which are also associated with the endosperm cell wall of the grain. In wheat, rye and triticale, arabinoxylans are the major NSP (Choct et al., 1995; Choct and Annison, 1992), while in barley and oats, β-glucan are the major NSP (Rotter et al., 1990; White et al., 1983). NSP, as an antinutritional factor, can not be hydrolyzed by enzymes that are produced endogenously by the bird. The antinutritional effect can be explained on the basis that these NSP may prevent access of endogenous enzymes to the nutrients contained within grain cells. The other prevailing explanation is that some of the cell wall NSP of these cereals dissolve in the digestive tract and form high molecular weight aggregates while increase viscosity (White et al., 1981). The high viscosity of the digesta reduces the passage time and impairs diffusion of digestive enzymes to their substrates and the mixing with gut content (Antoniou et al., 1981; Antoniou and Marquardt, 1982). Studies have shown that these viscous indigestible polysaccharides cause an enlargement of digestive organs, such as the pancreas (Marquardt et al., 1996), as they

Table 7. Feed enzymes in poultry nutrition[1]

Substrate	Enzyme	Microorganisms used to produce enzymes
Protein (animal or plant)	Protease	Aspergillus oryzae
		Trichoderma viridae
Starch	Amylase	Aspergillus niger
		Aspergillus oryzae
		Bacillus licheniformis
Lipids	Lipase	-[2]
Carbohydrates		
Cellulose	Cellulase	Aspergillus candidus
Arabinoxylans	Xylanase	Trichoderma viridae
β-glucans (barley, oats)	β-glucanase	Trichoderma viridae
		Humicola insolens
		Aspergillus ficuum
α-Galactosides	α-Galactosidases	Saccharomyces
		Cerevisiae
Phytic acid	Phytase	Aspergillus sydowl
		Bacillus subtilis

[1]Modified from Puchal and Mascarell (1999) and Ferket (1993).
[2] Data not available.

Figure 2. Classification of non-starch polysaccharides in vegetable feeds



Modified from Puchal and Mascarell (1999).

produce more intestinal enzymes to compensate for the digestive impediment. This viscous gel interferes with the digestion and absorption of nutrients in the cereal grains as well as with the digestion and absorption of nutrients found in the other ingredients of the ration. These nutrients can be less available to the chick because of poor digestibility, which in turn leads to poor performance.

Enzymes have been developed to reduce the negative effects of NSP and improve the feeding value of cereal-based diets. Xylanase and β-glucanase are the enzymes most effective for supplementing cereal-based diets. Studies have shown that application of xylanases and β-glucanases in cereal-based diets improved bird performance and increased nutrient digestibility (Petterson et al., 1990; Bedford and Classen, 1992; Friesen et al., 1992; Marquardt et al., 1994).

It is generally conceded that the improvement in performance in relation to enzyme supplementation is not due to complete hydrolysis of the polysaccharides and subsequent absorption of the released sugar (White et al., 1983; Chesson, 1987), but that the response is due to enzyme break down of the arabinoxylans and β-glucans into smaller polymers (Annison and Choct, 1991), and therefore, alter the ability of these polysaccharides to form highly viscous solutions that inhibit nutrient diffusion and transport.

c) Use of phytase to release bound phosphorus

Phytate P is either unavailable or poorly utilized by monogastric animals due to insufficient quantities of endogenous phytase (Nelson, 1967). The availability of phytate P in feed ingredients of plant origin ranges from 0% (Nelson, 1967) to more than 50% with a

wheat, corn, and soybean meal diet (Temperton and Cassidy, 1964). Guenter (1997) suggested that the inconsistent results depend on the level of calcium in the diet, type of ingredient, level of inorganic phosphorus (Temperton and Cassidy 1964), age of the animal, and level of vitamin D_3 (Edwards, 1983).

The increasing environmental awareness leads to increasingly stringent legislation, which forces poultry producers to reduce the environmental pressure of animal manure, particularly in regard to nitrogen and phosphorus loads. A commercial phytase produced by the fungus *Aspergillus ficuum* was introduced into the market in the early 1990s (Ravindran et al.,1995), and was developed specifically to increase the digestibility of phosphorus from vegetable materials. It has been well documented that phytase is effective in releasing a significant portion of the P bound in phytate to make it available to the broiler. Simons et al. (1990) reported that microbial phytase supplementation of a low phosphorus maize-soybean diet increased the availability of phosphorus to over 60% an increase of about 10%. Many other experiments (Denbow et al, 1995; Kornegay et al., 1996; Sebastian et al., 1996) supported this result showing improvements in phosphorus availability resulting from phytase supplementation at a range of 20% to 40%.

Phytase not only can release the bound phytate phosphorus but also has additional benefits to the animal. Yi et al. (1996) reported a 1 to 2% improvement in amino acid digestibility when phytase was added to a turkey diet. Furthermore, the AMEn value increaseed. Namkung and Leeson (1999) reported that phytase supplementation resulted in 1% increased in AMEn compared with the control diet, and Ravindran and Bryden (1997) showed a 3.5% improvement from 3109 kcal kg^{-1} to 3217 kcal kg^{-1}.

There is ample evidence to indicate that phytase supplementation improves the availability of mineral such as calcium (Schöner et al., 1991; Sebastian et al., 1996) and zinc (Sebastian et al., 1996) to broiler chickens.

Phytase supplementation also improves growth rate and feed conversion ratio (Simons et al., 1990; Denbow et al., 1995; Kornegay et al., 1996; Sebastian et al., 1996). Simons et al. (1990) suggested that the improved chick performance may be due to the release of minerals and trace elements from complexes with phytic acid, or the utilization of inositol by the animal after the hydrolysis of phytic acid to inositol and inorganic P, or a possible increased starch and amino acids digestibility.

d) Enzyme application in canola meal

NSP and phytate are the most important antinutritional factors in canola meal. The NSP component of canola meal consists mainly of arabinose, xylose, mannose, galactose, glucose and uronic acids. Arabinose, glucose and uronic acids are predominant (Simbaya, 1996; Table 8). High levels of uronic acid and glucose residue indicate that pectic-type substance (ie rhamnogalacturonans with attached side-chain subunits) and cellulose are the major polysaccharides in canola meal (Slominski and Campbell, 1990). Pectins also contain a relatively high proportion of the neutral sugars arabinose, xylose, galactose and rhamnose, and the bulk of the arabinose and galactose not associated with the pectic substances was derived from arabinan and/or arabinogalactan (Siddiqui and Wood, 1977). Pectinase,

Table 8. The component of total non-starch polysaccharides (mg g^{-1} DM)

	Yellow commercial meal	Brown commercial meal
Rhamnose	2.62	1.94
Fucose	2.62	2.21
Arabinose	47.58	46.75
Xylose	21.24	20.01
Mannose	4.66	4.5
Galactose	19.23	18.05
Glucose	74.69	65.01
Uronic acids	64.18	51.24
Total NSP (%)	**23.68**	**19.37**

Simbaya (1996)

α-galactosidase and cellulase are considered as the most appropriate enzymes to be selected for canola meal to hydrolyze NSP component, whereas phytase can be selected to hydrolyze phytate.

There are several studies that have indicated that carbohydrase or protease supplementation to canola based diets improve the feed conversion ratio (Simbaya et al. 1996; Guenter et al., 1995; Slominski, 1997). Other experiments showed a tendency to increase body weight gain and improve feed utilisation with addition of carbohydrase and protease (Alloui et al., 1994; Sosulski et al., 1990). Research (Nernberg, 1998) with phytase supplementation also shows a significant improvement in chick performance resulting from increasing phytate digestibility in canola meal basal diets. However, there were some limitations with exogenous enzyme supplementation. First, the quick passage rates for chicken, approximately 2-4h (Bedford and Schulze, 1998) is not enough time for the enzyme to work in the gut. Secondly, the optimum pH for exogenous enzyme to work efficiently was around 5 ~ 7, thus the activity of exogenous enzyme can be reduce by the low pH in the gizzard (pH 2.0).

Zyla and Koreleski (1993) and Nernberg (1998) demonstrated that phytate can be completely hydrolyzed by *in vitro* phytase treatment. *In vitro* experiments have also indicated that supplementation of fibrolytic enzymes to canola meal reduced the NSP level (Slominski and Campbell, 1990; Alloui et al. 1994) and increased the protein solubility (Alloui et al. 1994). These results suggest that under ideal conditions the enzymes are very effective.

Slominski et al. (1999) reported that *in vitro* enzyme (α-galactose, cellulase and phytase) pretreatment of canola meal decreased total NSP level and increased the soluble NSP content. When feeding this enzyme pretreated canola meal to rats, a significant improvement in feed conversion ratio was observed in week 3 and 4. As compared to the control diet, a significant improvement in total NSP, soluble NSP, oligosaccharide and phytate digestibility was observed for the enzyme pretreated meal. This suggested that *in vitro* enzyme pretreatment could reduce antinutritional factors and improve the nutrient digestibility. The mechanism of enzyme supplementation benefits may be the depolymerization of the NSP structure and releasing of the nutrients that were trapped in the cell wall, thereby the nutrient digestibility or availability was improved (Bedford and Schulze, 1998).

e) Enzyme additive effect

It is well known that enzymes have additive effects. To reduce the viscosity caused by the water-soluble arabinoxylans in wheat or rye may depend on the presence of not only endo-xylanase but also arabinofuranosidase, ß-glucanase, acetylxylan esterase, and feruloyl esterase, in the preparation (Marquardt, 1997).

Simbaya et al. (1996) demonstrated that there were additive effects of protease, carbohydrase and phytase enzymes when added to canola meal diets. Phytase supplementation slightly improved the feed efficiency. When phytase acted in concert with either protease G or carbohydrase G there was a progressive improvement in feed efficiency. Slominski et al. (1997) also demonstrated that a carbohydrase blend tended to show an improvement in weight gain (360g vs 378g) and feed to gain ratio (1.69 vs 1.65).

However, fortification of a blend of carbohydrase with phytase and protease enzyme resulted in further improvement ($P < 0.05$) in weight gain (360 vs 385) and feed efficiency (1.69 vs 1.64).

Ravindran et al. (1999) confirmed that it was advantageous to include both phytase and xylanase in wheat-based broiler diets. These two feed enzymes could facilitate each other's activity by providing greater substrate access and thereby further reducing the antinutritive properties of phytates and NSP. Validation of possible mechanisms contributing to the observed complementary action between phytase and xylanase in wheat-based diets is required.

f) Problem associate with enzyme utilization

A major advantage in using enzyme supplementation in poultry diets is that ingredients that are otherwise not used, or included only at low levels, may be utilized to a greater extent without loss of performance and at lower costs than conventional ingredients.

A problem associated with enzyme supplementation of monogastric diets is the relatively short transit time of feeds through the gastro-intestinal tract. Thus the enzymes need to be present in sufficient quantities, act rapidly and be resistant to the conditions that exist in the gastro-intestinal tract.

g) Enzyme supplementation effect on feed intake

Researches with barley, wheat, rye and oats based diets demonstrated that supplementation of xylanase and β-glucanase can decrease the digesta viscosity, increase

feed passage rate, and thus increase feed intake (White et al. 1981; Hesselman et al. 1982; Hesselman and Aman 1986; Petterson et al., 1990; Rotter et al., 1989).

The protein digestibility of canola and soybean meals, as protein supplements in poultry diets, is a very important factor for estimating the nutritive value of these meals. Protease can be supplemented to canola and soybean meal and attempts to increase the protein digestibility.

Sebastian et al. (1994) has reported that a protease supplementation in a corn/soybean meal based diet resulted in reduced feed intake of broilers. However, Simbaya (1996) found that a substantial reduction in feed intake was observed in laying hens fed canola meal diets containing a high level (0.5g kg^{-1}) of a protease supplement. The reason is unclear and needs to be investigated. Simbaya et al. (1996) suggested that either high dietary protein or protease supplemented diets result in a surplus supply of amino acids in the blood stream that may reduce appetite and consequently result in a depression in feed intake or the excessive supply of amino acids results in an elevated utilization of energy, depressing feed intake. However, more research is needed to explain this phenomenon.

In poultry, feed intake can be controlled by dietary energy, protein, amino acid profile, weight and volume. It is generally recognized among poultry nutritionists that a primary determinant of feed intake is the energy concentration of the diet. As the level of metabolizable energy in the diet increases or decreases, food intake changes inversely, although the rate of adjustment is not always sufficient to keep energy intake constant (Denbow, 1994).

Protein level and amino acid profile in the diet are also involved in the regulation of feed intake of birds. Almquist (1954) reported that feeding an amino acid deficient diet to chickens reduced food intake. Chicks fed either an amino acid unbalanced diet or an excess of amino acids reduced feed intake (Wethli et al. 1975) and changed the plasma amino acid profile of chickens (Denbow, 1994).

Li and Anderson (1983) concluded that the plasma and tissues free amino acid pools fluctuated, amino acids might either directly, or indirectly act as neurotransmitter precursors in the brain and alter neurotransmission in the appetite control center.

3. MANUSCRIPT I

IMPROVEMENT OF THE NUTRITIONAL VALUE OF CANOLA MEAL BY USING AN ENZYME PRETREATMENT METHOD

Abstract

This research was designed to explore the potential for improving the nutritive value of canola meal (CM) by pretreatment with a combination of enzymes during the canola seed crushing process. Twelve (12) of carbohydrase-like (ie., α-galactosidase, pectinase, cellulase) and two phytase enzymes were evaluated *in vitro* for their ability to hydrolyze oligosaccharides and phytate and to depolymerize the non-starch polysaccharides (NSP) of canola meal. When enzyme blends were used in laboratory incubation trials with canola meal it was demonstrated that under optimum moisture conditions (80%) a high degree of hydrolysis of phytate (66-100%) and to a lesser degree NSP (11-20%) occured. Complete hydrolysis of oligosaccharides was achieved using enzyme blends. In preparation for *in vivo* analyses a large quantity of desolventized CM was obtained from a commercial crushing firm, and laboratory incubation trials using four enzyme blends were conducted at existing moisture (16-20%) and temperature (45°C declining to 22°C) conditions. In contrast to the results at optimum (80% moisture) incubation conditions, little or no hydrolysis of phytate, oligosaccharides or NSP occurred, probably due to the low moisture conditions. However, when the enzyme pretreated meals were fed (30% of diet) to 4-day old broiler chickens for a 2-week period, improvements in growth performance relative to a control diet were noted. The results showed that live weight gain increased ($P<0.05$) from 392g (no enzyme) to 414g by one enzyme blend and feed conversion ratio improved ($P<0.05$) by 1.9%. Compared with control, three enzyme blends improved ($P<0.05$) ileal protein (77.3 vs 80.3%) and phytate (29.0 vs 41.5%) digestibilities and two enzyme blends improved ($P<0.05$) ME (2865 vs 3017 kcal/kg). There were no improvements with one enzyme blend relative to the control

diet. The study indicated that there is potential for improvement of the quality of CM by enzyme pretreatment. However, the relatively low moisture content in desolventized meal would appear to limit the effectiveness of pretreatment of CM during the crushing process and further research is needed to realize optimum effects of added enzymes.

INTRODUCTION

Canola meal, containing 36-40% protein, is considered to be a good source of protein supplement in poultry diets. However, several anti-nutritional factors, such as high fiber level, glucosinolate content, aromatic choline esters and high phytate level, result in lower amino acids availability and metabolizable energy for canola meal than soybean meal (Bell, 1993). These limitations prevent this ingredient from being fully utilized in poultry nutrition.

According to Slominski (1993) the high fiber level is considered one of the greatest restrictions to canola meal use in broiler diets. The dietary fiber components of canola meal include lignin associated with polyphenols, non-starch polysaccharide (NSP), cell wall protein and minerals associated with the fibre components (Simbaya, 1996).

Various attempts have been made to improve the value of canola meal by reducing its high fibre content. These include dehulling of the canola seed (Zuprizal and Chagneau, 1992; Leslie et al., 1973), breeding for low fibre content cultivars (Newkirk et al., 1997; Simbaya et al., 1995; Slominski, 1994) and enzyme supplementation (Alloui et al. 1994; Guenter et al., 1995; Simbaya et al., 1996; Slominski et al. 1997).

Exogenous enzymes have been used in poultry feed for a few years (Bedford, 1995; Marquardt et al., 1996; Annison and Choct, 1991; Guenter, 1997; Chesson, 1993). They are considered to have the greatest potential to eliminate antinutritional factors that hinder nutrient availability. Non-starch polysaccharides and phytate in feedstuffs are the main targets of commercial feed enzymes (Bedford and Schulze, 1998). Feed enzyme

supplementation can improve the NSP and phosphorus availability, reduce the negative impact of these indigestible residues and improve animal performance (Annison and Choct,1991; Sebastian et al., 1998). With xylanases, β-glucanases and phytase, the greatest improvements were achieved in poultry feeds containing wheat and barley (Choct et al., 1995; Marquardt et al.,1996; Bedford and Schulze, 1998).

Although feed enzymes have been used for a long time in poultry feed, most of the studies have focused on barley and wheat with xylanases and β-glucanases and corn/soybean meal diets with phytase. Most investigations (Slominski and Campbell, 1990; Simbaya et al., 1996) have indicated that enzyme supplementation of canola meal does not produce a significant improvement in broiler chick performance. In these experiments, the enzyme was directly added to the diet and usually resulted in no significant improvement (Simbaya et al., 1996; Alloui et al., 1994). There are two main limitations to the method of directly adding enzyme. First, the quick passage rate (ingestion to excretion) for chicken, approximately 2-4h (Bedford and Schulze, 1998), does not allow enough time for the enzyme to work effectively. Second, when exogenous enzyme having an optimum pH of 5 ~ 7 pass through the digestive tract, and encounter the low pH (pH 2.0) in the gizzard, the enzyme activity may be reduced.

Zyla and Koreleski (1993) reported that phytate in canola meal was completely hydrolyzed *in vitro* when canola meal was pretreated with crude phytase, and Newkirk and Classen (1998) reported similar results. These results suggest another opportunity for improving the nutritive value of canola meal. The process of producing canola meal was considered to offer some potential for pretreatment. The high moisture (15-20%) and

temperature (100°C) in the canola meal desolventizing process (Canola Council of Canada, 1997) may offer an environment for the enzyme to work.

The major objective of this study was to explore the potential for improving canola meal utilization through enzyme pretreatment and to identify the best enzyme blend to reduce the antinutritional factors. The second objective was to apply the best enzyme blend to a practical situation by mimicking the canola meal processing condition and investigating how pretreated canola meal during this process would affect chicken performance, phytate and NSP hydrolysis, ileal protein availability and energy availability.

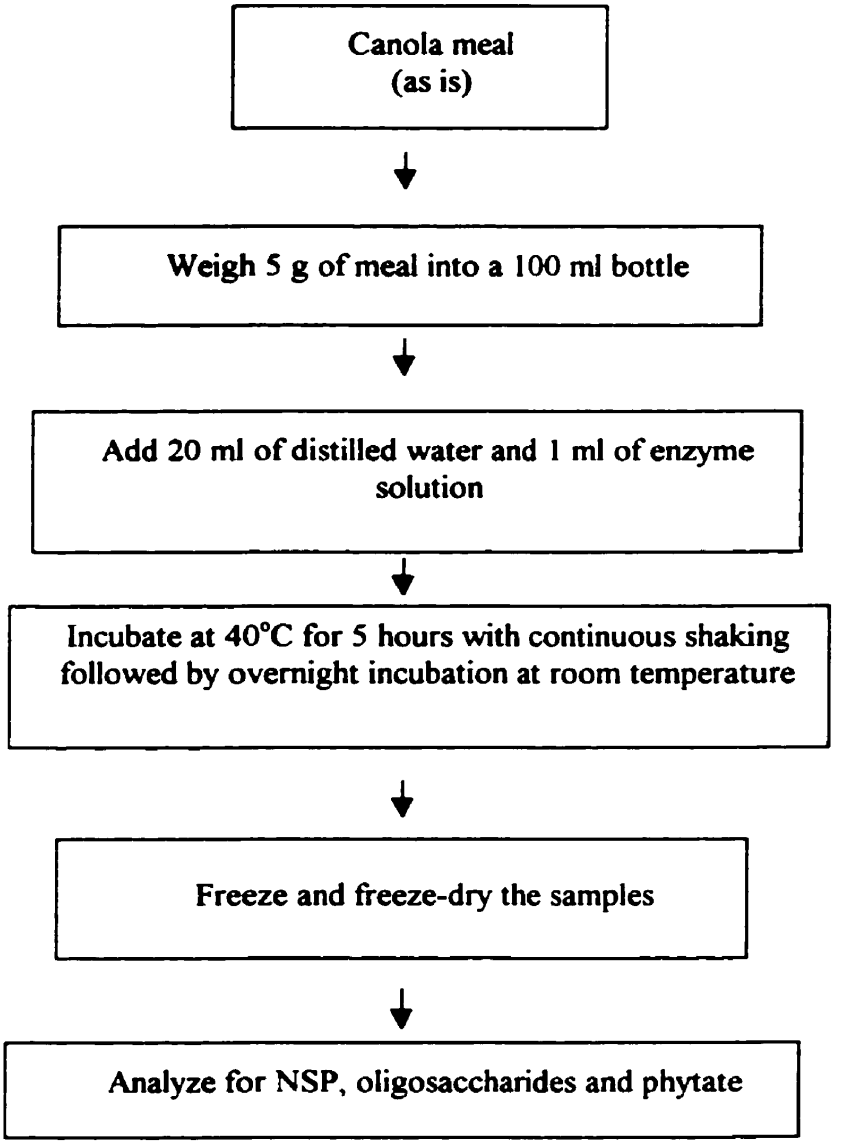
MATERIALS AND METHODS

IN VITRO STUDY

Canola meal incubation study

In the standard assay procedure (Figure 3), 5 g of canola meal was weighed into a 100ml Erlenmeyer flask and mixed with 20ml of distilled water and enzyme. The enzyme was supplemented at level 0.1% with carbohydrase or 0.02% with phytase A or 0.05% with phytase B in 1ml of distilled water for single enzyme trials, and with 0.1% total carbohydrases plus phytase in 1mL of distilled water for enzyme blend trial. The samples were incubated for 5hrs in an incubator-shaker (200rpm) at 40°C followed by overnight incubation at room temperature (22°C). Following freeze drying, the samples were analyzed for NSP, phytate and oligosaccharide content.

Figure 3. *In vitro* procedure for incubation of canola meal with exogenous enzyme



Twelve carbohydrase-like (ie., α-galactosidase, pectinase, cellulase) and two phytase enzymes (Table 9) were evaluated *in vitro* for their ability to reduce the NSP fraction and phytate phosphorus in canola meal (trials 1, 2 and 3). Selected enzyme blends were evaluated for their ability to maximize phytate, oligosaccharide and NSP hydrolysis (trial 4). Enzymes for each blend were selected on the basis of their ability to hydrolyze NSP and phytate content, specific enzyme activity (ie., α-galactosidase, pectinase, cellulase, hemicellulase and phytase) and source (manufacturer). There were three selected enzyme blends (enzyme blend I, II and III) to be used both for *in vitro* and *in vivo* evaluation. One enzyme blend (enzyme blend IV) consisted of carbohydrase D and an experimental phytase C that were not previously tested *in vitro*.

The composition of enzyme blends I, II, III and IV are showing in Table 10. The enzyme blends were supplemented at 0.1%level.

***In vitro* protein digestibility studies**

The method used for *in vitro* protein digestibility evaluation involved a two step digestion using pepsin, followed by pancreatin as recommended by Gauthier et al. (1982) and Kennedy et al. (1989). The method that was used in this research is summarized in Figure 4 and is described as follows:

Five grams of sample were weighed into a 100ml Erlenmeyer flask and mixed with 25ml distilled water. Samples were boiled in a water bath for about 5min and cooled to room temperature. Twenty-five milliliters of 0.2M HCl/108mM NaCl solution and 500mg of pepsin (P7000, Sigma, St. Louis, MO, U.S.A.) were added, and then contents were shaken

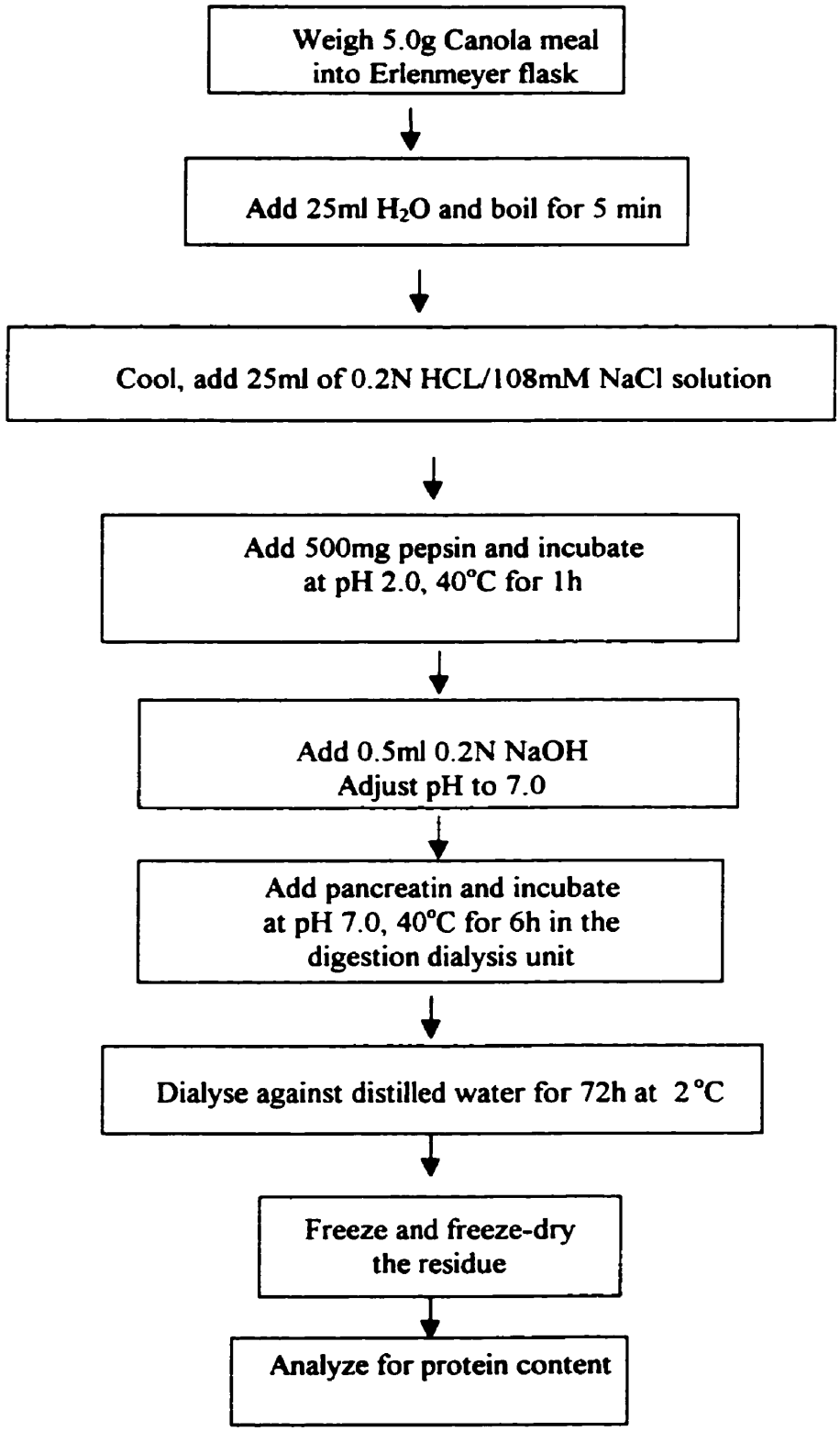
Table 9 Experimental enzyme preparations used in the *in vitro* and *in vivo* studies

Enzyme name	Main activity
Carbohydrase A	Alpha-galactosidase, pectinase
Carbohydrase B	Cellulase
Carbohydrase C	Cellulase
Carbohydrase D	Cellulase, hemicellulase
Carbohydrase E	Cellulase
Carbohydrase F	Pectinase
Carbohydrase G	Cellulase
Carbohydrase H	Glucanase, cellulase
Carbohydrase I	Cellulase
Carbohydrase J	Xylanase, cellulase
Carbohydrase K	Hemicellulase
Carbohydrase L	Cellulase
Phytase A	Phytase
Phytase B	Phytase
Phytase C	Phytase

Table 10. The composition of enzyme blends

Enzyme blend	Enzyme composition
Enzyme blend I	Carbohydrase A (0.05%) Carbohydrase B (0.03%) Phytase A (0.02%)
Enzyme blend II	Carbohydrase A (0.05%) Carbohydrase B (0.015%) Carbohydrase C (0.015%) Phytase A (0.02%)
Enzyme blend III	Carbohydrase A (0.03%) Carbohydrase D (0.03%) Phytase B (0.04%)
Enzyme blend IV	Carbohydrase D (0.05%) Phytase C (0.05%)

Figure 4. *In vitro* procedure for determination of digestible protein content of enzyme-treated canola meal



for 1h at 40°C in an environmentally controlled incubator-shaker (New Brunswick Scientific Co., Inc. Edison, NJ. U.S.A.). The pH of the solution was adjusted to 7 with 2.5ml of 2.0M NaOH. The contents were transferred into pre-soaked dialysis tubes (Spectrum, Houston, TX, U.S.A.) with a molecular weight cut off value of 12000-14000 and the flask was washed with 20ml of 0.1 M phosphate buffer to wash the container. One ml of phosphate buffer solution containing 50mg of pancreatin (P1750, 4 x U.S.P.; Sigma Chemical Co., St. Louis, MO, U.S.A.) was then added and tubes were closed allowing for a small air gap in the tube to facilitate continued mixing of the contents (Kennedy et al., 1989).

The tubes were attached to the metal frame mounted on a thermally controlled water bath (Bodine Electric Co., Chicago, Illinois, U.S.A.) filled with 0.05 M phosphate buffer pH 7.0. Digestion was allowed to process for 6hrs at 40°C with the metal frame rotating at approximately 20rpm. After digestion, the buffer was replaced with ice-cold distilled water to cool the system to about 2°C to terminate the enzyme activities. The dialysis continued for 72 h at 2°C with continuous rotation of the tubes. Dialysis water was changed twice a day. Following dialysis, the samples were transferred to plastic bags, frozen, freeze-dried and analyzed for crude protein.

Digestible protein content was calculated using the following equation

$$\text{Digestible protein (\%)} = \frac{(Ws \bullet CPs)-(Wr * CPr)}{(Ws * CPs)} \times 100$$

Ws: Sample weight

CPs: Protein content in sample

Wr: Residual sample weight

CPr: Residual sample protein content

IN VIVO STUDY

Sample preparation: enzyme pretreated canola meal

Canola meal was provided by CanAmera Foods (Altona, MB, Canada). The high moisture (15.9%) canola meals were obtained directly from the desolventizer. In order to keep the temperature, the sample were well sealed in insulated containers. Following sampling, the high moisture canola meal (45°C) was supplemented with 0.1% exogenous enzyme blend I or IV (Table 11) and mixed on a continuous basis for 30min. Following overnight incubation at room temperature, the meals were put under a fume-hood to dry to 12.8% moisture content. Conventional canola meal from the same batch was obtained at a moisture level of about 11%. The conventional meal was heated to 45°C, and water was added to increase the moisture to 20% and supplemented with 0.1% exogenous enzyme blends I, II, III or IV (Table 11) and mixed on a continuous basis for 30min. Following overnight incubation at room temperature, the meals were put under a fume-hood to dry to 12.8% moisture content. All the meals were reground to pass through a 2mm sieve prior to using for diet preparation. The NSP, phytate and oligosacchairdes contents were determined.

Animal and diets

Two week feeding trials starting with 4-day-old broiler chicks were employed to study the effect of enzyme pretreated canola meal.

Table 11. Composition of enzyme blends used for pretreatment of canola meal

Treatment		Enzyme blend	Enzyme component
1	Control	None	
2	High moisture meal[1]	Blend I	Carbohydrase A Carbohydrase B Phytase A
3	High moisture meal	Blend IV	Carbohydrase D Phytase C
4	Low moisture meal [2]	Blend I	Carbohydrase A Carbohydrase B Phytase A
5	Low moisture meal	Blend II	Carbohydrase A Carbohydrase B Carbohydrase C Phytase A
6	Low moisture meal	Blend III	Carbohydrase A Carbohydrase D Phytase B
7	Low moisture meal	Blend IV	Carbohydrase D Phytase C

[1] Moisture content 15.9% at time of sampling
[2] Moisture content 11.0% at time of sampling. At enzyme pretreatment, the moisture content increased to 20%.

One-day-old vaccinated (Marek's) male broiler chicks were obtained from a commercial hatchery. The birds were housed in Jamesway chick batteries and fed a commercial chick starter diet containing 20% protein from day 1 to day 4. On day 4, birds were individually weighed and placed into narrow weight classes. Groups of fives birds were then assigned to pens in Petersime brooder batteries such that all pens had a similar initial weight. Each treatment was randomly assigned to 10 replicates (pens) of five bird each. From Day 4 to 18, the birds were fed experimental diets that were in a mash form. The study involved 6 diets of pretreated canola meal and a control diet without enzyme (Table 12). All diets were formulated according to NRC (1994), but were marginal in available amino acids and non-phytate P (Table 12) to provide an opportunity for a measurable response to enzyme addition. Chromic oxide was added to the diets at the level of 0.35% for aiding in the determination the digestibility of protein, NSP, phytate, oligosaccharide and the AMEn value. To eliminate the negative effect of wheat, xylanase and β-glucanase enzymes (xylanase 500u $kg^{-1}$and β-glucanase 200u kg^{-1}) were added to all diets.

The chicks were fed the experimental diets for 14 days. They had free access to water and feed and continuous light. On day 7 and day 14 of the experiment, the birds were fasted for 4 h and group weighed. Feed intake also was recorded. On day 14 of the experiment, the excreta were collected for determination of phytate phosphorus, NSP and AMEn. On day 15 of the experiment, 6 birds per treatment (one bird per pen randomly selected from each of six replicate) were killed by cervical dislocation. Ileal contents were collected from the

Table 12. Composition and calculated analysis of experimental diets

	Diet 1 – Diet 7
Ingredient %	
Soybean meal	2.5
Canola meal[1]	30
Wheat	58.95
Limestone	1.7
$CaHPO_4$	0.68
Lysine	0.12
Vitamin premix[2]	1
Mineral premix[3]	0.5
Vegetable oil	4.2
Chromium oxide	0.35
Calculated composition %	
Metabolizable energy (kcal/kg)	2908
Crude protein	21
Calcium	1
Available phosphorus	0.35
Methionine	0.42
Methionine & cystine	0.88
Tryptophan	0.25
Lysine	1
Threonine	0.73
Isoleucine	0.68
Leucine	1.31
Arginine	1.08
Valine	0.91

[1] Enzyme pretreated canola meal as specified in Table 11

[2] Provided (per kg of diet): Vitamin A, 8250IU; Vitamin D_3, 1000IU; Vitamin E, 10.9IU; Vitamin B_{12}, 0.0115mg; Vitamin K, 1.1mg; Niacin 53.3mg; riboflavin, 75mg; choline chloride, 1020mg; Ca-Pantothenate, 11mg and Biotin; 0.25mg.

[3] Provided (per kg of diet): Zn, 50mg; Mn, 55mg; Fe, 80mg; Cu, 5mg; Se, 0.1mg and I, 0.36.

last half of the ileum and contents from 2 birds were pooled (3 replicates/treatment), the samples were lyophilized and analyzed for protein.

CHEMICAL ANALYSIS

Diets and excreta samples were ground using a coffee grinder to pass through 1mm sieve size. All samples were analyzed in duplicate for crude protein (AOAC, 1990), chromic oxide content (Williams et al., 1962), NSP (Slominski, 1994), phytate phosphorus (AOAC, 1990), oligosaccharides (Slominski and Campbell, 1991) and AMEn (Hill et al., 1960).

CALCULATIONS AND STATISTICAL ANALYSIS

The digestibility of protein, NSP, phytate and oligosaccharide were calculated on dry matter basis using the following equation:

$$\text{Digestibility} = \left(1 - \frac{Cr_2O_{3\,diet} * X_{digesta}}{Cr_2O_{3\,digesta} * X_{diet}} \right) \times 100$$

Where $X_{digesta}$ and X_{diet} are concentration of a specific nutrient in the digesta and diet, $Cr_2O_{3\,digesta}$ and $Cr_2O_{3\,diet}$ are concentration of Cr_2O_3 in the digesta and diet.

AMEn was calculated by using the method of Hill (1960).

The results obtained from the chicken experiment were analyzed by the general linear model (GLM) procedure (SAS Institute Inc., 1990). A complete randomized design was used, a single pen representing the experimental unit (replicate). For the protein digesta,

there were 3 replicates per treatment. Mean differences were determined using Duncan's multiple range test (Duncan, 1955). Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

IN VITRO STUDY

Canola meal incubation study

The results of the *in vitro* incubation of canola meal with the single enzymes are shown in Tables 13, 14 and 15. Incubation of canola meal with various commercial enzymes resulted in varying degrees of NSP hydrolysis. It was shown that carbohydrase A, carbohydrase B and carbohydrase D gave the highest NSP reduction among the twelve commercial carbohydrases tested. The values of reduction for NSP were 15%, 9% and 8% for carbohydrase A, carbohydrase B and carbohydrase D, respectively. Both Phytase A and Phytase B show significant phytate reduction. The reduction of phytate was 40% and 80% respectively, when canola meal was incubated with phytase A and phytase B (Table 15). Based on these results, carbohydrase A, carbohydrase B, carbohydrase D, Phytase A and Phytase B were chosen for further testing in combination. Carbohydrase C was selected based on its major enzyme activity (cellulase) and its source (manufacturer).

The results of the *in vitro* incubation of canola meal with the enzyme blends (I, II and III) are shown in Table 16. All three enzyme blends (I, II and III) significantly reduce both NSP (13%, 11% and 21%) and phytate (66%, 68% and 96%) content, respectively.

Table 13. The effect of carbohydrase pretreatment of canola meal on non-starch Polysaccharide (NSP),NSP-glucose, phytate and oligosaccharide content (trial 1) (80% moisture content)

Enzyme	NSP	NSP-glucose	Phytate	Oligosaccharides
	----------(g/kg)----------			
None(control)	177.8 ± 1.3[a1]	65.8 ● 1.7[b]	26.4 ● 0.6[a]	21.5 ● 0.8[a]
Carbohydrase A	150.5 ± 0.7[d]	44.3 ± 1.1[d]	20.4 ± 1.2[b]	nd[2]
Carbohydrase D	162.7 ± 4.4[c]	60.2 ± 2.2[c]	26.6 ± 0.8[a]	21.1 ± 1.2[a]
Carbohydrase E	175.7 ± 0.9[a]	69.6 ● 0.6[a]	25.1 ± 1.2[a]	20.3 ± 1.6[a]
Carbohydrase K	170.5 ● 0.7[b]	68.6 ± 0.5[ab]	24.2 ± 1.8[a]	26.3 ± 2.3[a]

[1]Mean ± SD; Means within a column with no common superscript differ significantly (P<0.05)
[2] Not detected

Table 14. The effect of carbohydrase pretreatment of canola meal on non-starch Polysaccharide (NSP),NSP-glucose, phytate and oligosaccharide content (trial 2) (80% moisture content)

Enzyme	NSP	NSP-glucose	Phytate	Oligosaccharides
	----------(g/kg)----------			
None (control)	174.3 ± 2.3^{abc1}	72.6 ± 1.3^{ab}	26.2 ± 2.4^{a}	21.2 ± 1.1^{a}
Carbohydrase B	158.6 ± 1.4^{d}	68.3 ± 4.0^{ab}	25.8 ± 1.2^{a}	22.5 ± 0.6^{a}
Carbohydrase L	182.2 ± 2.0^{bcd}	76.9 ± 3.1^{a}	25.8 ± 2.3^{a}	21.4 ± 0.3^{a}
Carbohydrase C	169.3 ± 9.9^{abc}	73.0 ± 3.3^{ab}	25.2 ± 0.6^{a}	21.9 ± 1.2^{a}
Carbohydrase F	178.5 ± 3.7^{abc}	71.8 ± 4.4^{ab}	24.3 ± 2.8^{a}	-[2]
Carbohydrase G	173.0 ± 7.0^{abc}	67.1 ± 4.9^{b}	25.9 ± 1.3^{a}	-
Carbohydrase H	166.9 ± 4.5^{dc}	68.3 ± 2.8^{ab}	27.2 ± 0.7^{a}	-
Carbohydrase I	180.4 ± 0.6^{ab}	70.1 ± 2.8^{ab}	27.4 ± 0.2^{a}	-
Carbohydrase J	182.7 ± 3.8^{a}	72.9 ± 4.0^{ab}	26.0 ± 1.5^{a}	-

[1]Mean ± SD; Means within a column with no common superscript differ significantly ($P<0.05$)
[2] not measure

Table 15. The effect of phytase pretreatment of canola meal on non-starch Polysaccharide (NSP),NSP-glucose, phytate and oligosaccharide content (trial 3) (80% moisture content)

Enzyme	NSP	NSP-glucose	Phytate	Oligosaccharides
	----------(g/kg)----------			
None (control)	174.3 ± 2.3[a1]	72.6 ± 1.3[a]	26.2 ± 2.4[a]	21.0 ± 1.4[a]
Phytase A	175.4 ± 0.5[ab]	73.4 ± 1.8[a]	15.3 ± 0.4[b]	21.4 ± 0.4[a]
Phytase B	173.0 ± 0.7[b]	73.5 ± 2.6[a]	5.1 ± 2.4[c]	19.5 ± 2.4[a]

[1]Mean ± SD; Means within a column with no common superscript differ significantly (P<0.05)

Table 16. The effect of carbohydrase and phytase pretreatment of canola meal on Non-starch polysaccharide (NSP), NSP-glucose, phytate and oligosaccharide content (trial 4) (80% moisture content)

Enzyme blend	Composition	NSP	NSP-glucose	Phytate	Oligsaccharides
		(g/kg)			
None	Control	172.9 ± 0.0[a1]	66.4 ± 0.8[a]	25.8 ± 2.4[a]	21.0 ± 0.14
Blend I	Carbohydrase A Carbohydrase B Phytase A	150.5 ± 0.2[b]	49.2 ± 2.1[b]	8.7 ± 3.0[b]	nd[2]
Blend II	Carbohydrase A Carbohydrase B Carbohydrase C Phytase A	153.5 ± 3.3[b]	52.0 ± 2.0[b]	8.2 ± 0.6[b]	nd
Blend III	Carbohydrase A Carbohydrase D Phytase B	137.3 ± 2.6[c]	35.8 ± 0.1[c]	1.1 ± 0.6[c]	nd

[1]Mean ● SD; Means within a column with no common superscript differ significantly ($P<0.05$)
[2] Not detected

Among the enzymes tested in the current study, carbohydrase A was found to be the most effective enzyme to hydrolyze NSP. The combination of carbohydrase A, phytase B and carbohydrase D resulted in highest reduction of NSP (21%) and phytate (96%). All three enzyme blends plus one additional were chosen for further testing using an *in vivo* method.

High fiber and phytate content are considered to be the major anti-nutritional factors responsible for the restricted use of canola meal in poultry diets. Fiber may interfere with protein and mineral digestion, whereas phytate binds to divalent cations as well as protein and reduces the protein and mineral availability. During *in vivo* digestion only relatively small amounts of NSP (Slominski and Campbell, 1990) and phytate are hydrolyzed in the digestive tract. This is true even when exogenous carbohydrase and phytase enzymes are added to the diet (Simons et al., 1990; Slominski and Campbell, 1990). This can be partially explained by quick passage rate and poor conditions for enzyme action during digesta passage through the gastro-intestinal tract. To effectively overcome anti-nutritional effects of NSP and phytate, it is desirable to use enzyme pretreatment to maximize enzyme effect.

Zyla and Koreleski (1993) detected no phytate following incubation of canola meal with a mixture of phytase and acid phosphatase for 4h at pH 4.5 and 40°C. Nernberg (1998) also reported that no phytate could be detected in the sample when canola meal was incubated with phytase (Natuphos®) *in vitro*, suggesting complete phytate hydrolysis. The optimal *in vitro* incubation condition (pH4.5 ~ 5.5, 40°C and 83.3% moisture) used in the experiment by Nernberg (1998) may explain the extensive hydrolysis. Both Zyla and Koreleski (1993) and Nernberg (1998) used a low pH solution to hydrolyze the phytate. In our studies, however, the solvent was pure water at pH 7.0. According to Nernberg

(1998), the optimum pH for phytase activity is 5.5. At pH 7.0, the activity of phytase is only about 10-20% of maximum obtained at pH5.5. Therefore more time would be required to completely hydrolyze phytate. The current results are in agreement with Newkirk and Classen (1998) who reported that complete hydrolysis of phytate required approximately 23h *in vitro*. It appears that 70-80% of the phytate is readily degraded (about 30min-2h) but the remaining 20% is somehow protected and takes longer to hydrolyze. This supported the view that time during digestion is a serious constraint.

Phytase B was more efficient ($P<0.05$) than phytase A to hydrolyze phytate. This may suggest that the two phytases have different side enzyme activity. Newkirk and Classen (1998) demonstrated that the ability of phytase to act on phytate was depended on phytase in concert with other undetermined enzyme activity. Phytase B and phytase A derived from different bacteria may have different enzyme profiles.

The study with enzyme blends showed that there was an additive effect. There was only 40% reduction on phytate content when canola meal was incubated with phytase A. But when phytase A was used in concert with carbohydrases A and B (blend I) or with carbohydrases A, B and C (blend II), phytate hydrolysis was increase to 66% and 68%, respectively. Enzyme blend I and II did not show additive effect on NSP reduction. However, with enzyme blend III, phytase B plus carbohydrases A and D, showed an additive effect both for NSP and phytate. When used alone, carbohydrase A and carbohydrase D reduced NSP content15% and 8%, respectively. Phytase B reduced phytate content 80%. However, the combination of carbohydrases A, D and phytate B, reduced the phytate content by 96% and the NSP content by 21%. This additive effect may

suggest that enzyme blends can be more effectively used to reduce antinutritive factors than single enzymes. This is in accordance with previous experiments (Simbaya et al. 1996) that broiler chicken fed a blend of enzymes performed better than those fed individual enzymes. Since the reduction was much higher for phytate than for NSP, the NSP was much more difficult to hydrolyse than phytate. This is in agreement with the suggestion that cell wall degrading enzymes exert their effects by a relatively slow mechanism of surface peeling (Hotten, 1991), since most NSP exists in the cell wall.

A complete hydrolysis of oligosaccharides was achieved with all three enzyme blends (blend I, II and III). The oligosaccharides in canola meal may not reduce nutrient digestibility (Slominski et al. 1994), but excessive fermentation in the small intestine due to oligosaccharides may interfere with the normal physiological process of nutrient digestion (Misir and Marquardt, 1978). Furthermore, canola meal contains about 2.5% oligosaccharides. If all of the oligosaccharides were hydrolyzed and the monosaccharide used for energy this would result in an increase of 89 kcal ME/kg (0.025 x 3600 kcal of glucose) of canola meal or a 4.7% increase (Puchal and Mascarell, 1999).

In vitro protein digestibility

Five treatments were selected including single enzymes and/or enzyme blends to evaluate protein digestibility *in vitro*. There were no marked differences between *in vitro* protein digestibility of the control (no enzyme) and enzyme pretreated canola meals (Table 17).

Table 17. Effect of enzyme pretreatment of canola meal on in vitro protein digestibility (% dry matter)

Enzyme	Protein digestibility (%)
None (control)	66.9[1]
Carbohydrase A	66.8
Carbohydrase D	67.1
Carbohydrase A, Carbohydrase B, Carbohydrase D	67.3
Carbohydrase A, Carbohydrase B, Carbohydrase C, Phytase A	67.6
Carbohydrase A, Carbohydrase B, Phytase B	66.7
SEM	1.2

[1] No statistical significant differences were observed (P>0.05)

This result is in agreement with those of Serraino et al. (1985) who showed that phytase pretreated rapeseed meal did not improve the *in vitro* protein digestibility but it did change the rate of release of many amino acids. Newkirk et al. (1997) also reported that phytase pretreatment of canola meal does not improve its nutritional value for broiler chicks. However, theoretically, amino acids and small peptides bound to phytic acid in the form of protein-mineral-phytate complex (de Rham and Jost, 1979) are believed to obstruct or inhibit the enzymatic degradation of the protein (Singh and Krikorian, 1982).

IN VIVO STUDY

Sample preparation: enzyme pretreated canola meal

Enzyme pretreatment of canola meal did not reduce the NSP and phytate content of the meal (Table 18). The reason for the insignificant differences between control and pretreated canola meal is probably due to the moisture (16-20%) content employed in this study. Slominski et al. (1999) reported that NSP and phytate reduction are about 6% and 9% respectively, at 40% moisture content compared with 16% and 30%, respectively, at 80% moisture content.

Animal Trial

The effects of canola meal pretreated with different enzyme blends on feed intake, weight gain and feed conversion ratio of broiler chickens are shown in Table 19. There are no significant effects of enzyme blends on feed intake. Only enzyme blend III showed a

Table 18. Phytate and non-starch polysaccharide (NSP) content of enzyme pretreated canola meal(g/kg, as fed basis)

Treatment[1]	Phytate	Total NSP	Oligosaccharides
1 (control)	23.9 ± 0.38[2]	157.4 ± 5.9	22.6± 1.2
2 (Blend I)	23.4 ± 1.17	152.2 ± 2.8	22.1± 2.1
3 (Blend IV)	24.0 ± 1.72	152.0 ± 0.2	22.5± 0.8
4 (Blend I)	23.9 ± 0.45	158.1 ± 2.9	21.7± 0.9
5 (Blend II)	24.6 ± 2.58	162.3 ± 4.2	22.0± 0.5
6 (Blend III)	23.5 ± 1.91	163.2 ± 1.2	22.6± 1.8
7 (Blend IV)	25.2 ± 1.20	166.2 ± 3.6	22.2± 1.0

[1] See table 11 for treatment description

[2] Means ± SD

Table 19. Feed intake, body weight gain and feed conversion ratio of broiler chickens fed diets containing enzyme pretreated canola meal

Diet[1]	Feed intake (g/ bird/ 14days)	Body weight gain (g/ bird/ 14days)	Feed conversion ratio
1 (control)	610 ● 21[a]	392 ± 14[b2]	1.55 ● 0.02[abc]
2 (Blend I)	612 ● 33[a]	392 ● 18[b]	1.56 ± 0.02[ab]
3 (Blend IV)	612 ● 34[a]	390 ± 24[b]	1.57 ± 0.02[a]
4 (Blend I)	609 ± 31[a]	397 ± 21[ab]	1.54 ± 0.03[cd]
5 (Blend II)	619 ● 31[a]	402 ± 21[ab]	1.54 ± 0.03[bcd]
6 (Blend III)	628 ± 24[a]	414 ± 18[a]	1.52 ± 0.03[d]
7 (Blend IV)	613 ● 34[a]	390 ± 24[b]	1.57 ± 0.02[a]

[1] See table 12 for treatment description

[2] Means ● SD; Means within a column with no common superscript differ significantly ($P<0.05$)

significant ($P<0.05$) increased body weight gain compared to the control. This resulted in an improvement in the feed conversion ratio associated with this enzyme mixture.

The digestibilities of phytate, NSP and protein as well as the metabolizable energy (AMEn) as determined with excreta or ileum digesta are presented in Table 20. Generally, the addition of enzyme blend I, II and III improved ($P<0.05$) protein and phytate digestibility, and enzyme blend II and III also increased ($P<0.05$) the AMEn value of the diet. Enzyme blend III increased oligosaccharide digestibility. Enzyme blend IV had no effect on digestibility of protein, NSP and phytate and the AMEn level.

The animal trial demonstrated that performance of broiler chickens was improved by the enzyme blend pretreatment of canola based diets with enzyme blend III (carbohydrase A and D and phytase B). The low productive value of unsupplemented canola meal when given to broiler chickens has been attributed to high fiber and phytate content. The NSP fraction and phytate are not degraded sufficiently (Slominski and Campbell, 1990; Guenter, 1997) and would cause low available protein digestibility and ME in the meal, and thus interfere with nutrient absorption (Bell, 1993).

Improvements in performance of broiler chickens given canola-based diets supplemented with enzyme as observed in the present study are in agreement with results reported earlier (Guenter et al., 1995; Simbaya et al., 1996). The response of enzyme addition was, however, influenced by the combination of enzymes, and it was more pronounced with enzyme blend III. Enzyme blend III supplementation improved ($P<0.05$) the body weight gain by 5.6% and the feed conversion efficiency by 1.9%, but there were no significant

Table 20. Effect of enzyme pretreated canola meal on the apparent ileal protein digestibility,AMEn content and total-tract digestibility of phytate, NSP and oligosaccharides

Diet[1]	Protein (%)	Phytate P (%)	NSP (%)	Oligosaccharides (%)	AMEn Kcal/kg
1 (control)	77.3 ± 0.8^{dc2}	29.0 ± 6.8^{c}	19.4 ± 2.5^{cd}	54.2 ± 1.6^{b}	2865 ± 31^{b}
2 (Blend I)	81.8 ± 0.9^{a}	37.5 ± 4.1^{b}	22.1 ± 2.0^{bc}	57.3 ± 3.2^{b}	2853 ± 33^{b}
3 (Blend IV)	76.8 ± 1.5^{e}	28.7 ± 8.2^{c}	17.4 ± 2.6^{c}	55.3 ± 2.3^{b}	2825 ± 23^{b}
4 (Blend I)	79.5 ± 0.4^{bc}	36.5 ± 3.0^{b}	24.0 ± 2.3^{b}	56.3 ± 1.3^{b}	2802 ± 115^{b}
5 (Blend II)	80.1 ± 1.2^{abc}	39.2 ± 2.8^{b}	28.3 ± 1.8^{a}	56.1 ± 2.5^{b}	3017 ± 26^{a}
6 (Blend III)	81.3 ± 0.8^{ab}	48.9 ± 5.2^{a}	24.3 ± 1.2^{bc}	61.9 ± 1.6^{a}	3016 ± 12^{a}
7 (Blend IV)	79.0 ± 1.2^{dc}	29.6 ± 2.8^{c}	23.9 ± 3.0^{b}	56.2 ± 0.8^{b}	2849 ± 48^{b}

[1] See table 11 for treatment description

[2] Means ± SD; Means within a column with no common superscript differ significantly ($P<0.05$)

differences with the other enzyme blends (blend I, II and IV) on feed intake and feed conversion ratio. It is important therefore to select the most appropriate enzyme blends for a specific feed substrate. These figures show how an inappropriate enzyme blend can make little or no difference to bird performance, while careful screening to select the most effective enzyme blend can reap significant improvements in feed conversion ratio and growth. The improvement with broiler chicken performance for enzyme blend III can be ascribed to the improvement of the digestibility of phytate and increasing AMEn value. Since phytase B in blend III was more efficient than phytase A, it may render enzyme blend III better than other enzyme blends (blend I, II and IV).

Enzyme blend I, II and III improved the phytate digestibility by 26%, 35% and 67%, respectively. The improved P digestibility is in agreement with data presented by Nelson et al., (1968) Denbow et al., (1995) and Sebastian et al., (1996). Improvements in phosphorus availability resulting from phytase supplementation are generally reported to be in the range of 20-40% (Sebastian et al., 1998), which is similar to enzyme blend III in the current study.

The improvement of protein digestibility and energy value of canola-based diets is in agreement with those of Cowan et al. (1996). The improvement in the protein digestibility and AMEn with enzyme blend III is 5.1% and 5.2%, respectively. Enzyme blend II supplementation improved the protein digestibility by 3.5% and AMEn by 5.3%. However, enzyme blend I pretreated high moisture canola meal and conventional canola meal improved the protein digestibility by 5.8% and 2.8%, respectively, but no improvement was observed for AMEn. No improvement for protein digestibility and AMEn was found with

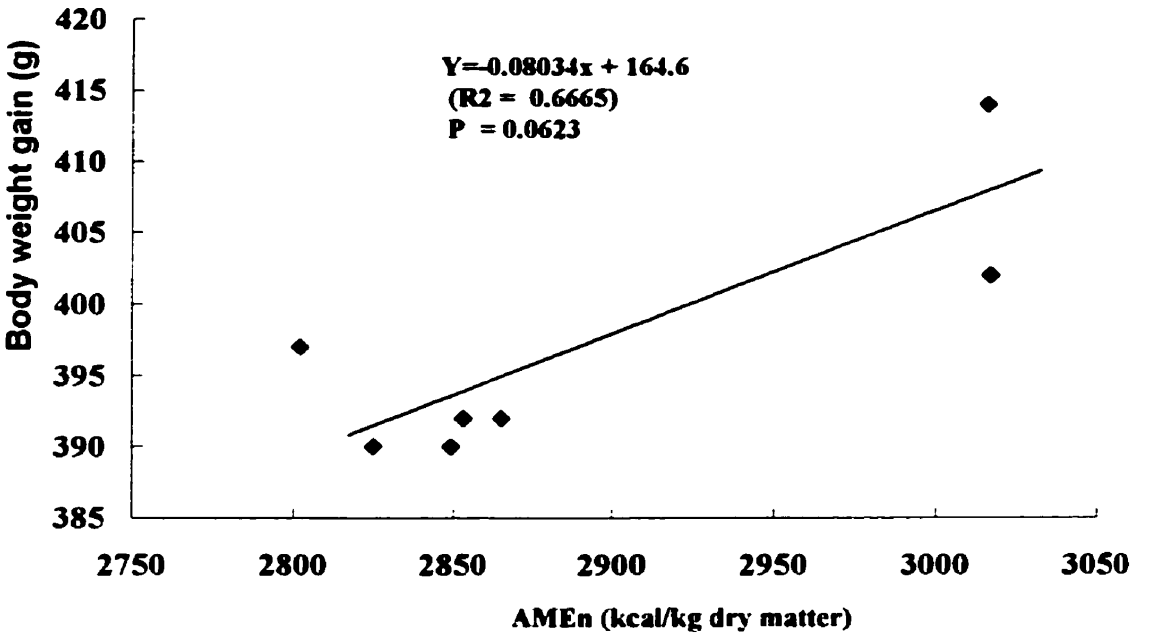
enzyme blend IV pretreated high moisture and conventional canola meal. Significant responses in the apparent nutrient digestibility and AMEn following the addition of enzymes have long been recorded in cereal-based diets (Rotter et al., 1990; Annison, 1992; Friesen et al., 1992; Choct et al., 1995). Annison (1992) reported an increase in AMEn of a wheat-based diet at a range of 6 to 9%, similar to our study.

The reason for the improvement in nutrient digestibility and AMEn value is likely related to increase the digestibility of phosphorus. Since phytate has the potential to form complexes with protein (Cheryan, 1980), which can negatively affect availability or digestibility of amino acids and protein (Namkung and Leeson, 1999). Furthermore, phytate is also able to bind endogenous proteases with a subsequent decrease in the digestibility of protein and amino acids (Singh and Krikorian, 1982). The diet with phytase supplementation had a higher AMEn and protein digestibility compared with the control diet. This also supports the results of Namkung and Leeson (1999), Ravindran and Bryden (1997) and Yi et al. (1996).

Other reasons for the improved nutrient digestibility and AMEn value with enzyme-supplementation is that the carbohydrases may break down the cell wall allowing the endogenous digestive enzymes to access cell content.

Regression analysis of the energy in the seven feeds on BW showed that performance was closely related to AMEn (Figure 5) with $R^2 = 0.66$ ($P = 0.06$).

Figure 5. Relationship between AMEn content and body weight gain of broiler chicken



CONCLUSION

In vitro enzyme treatment of canola meal under 80% moisture conditions was found to effectively reduce most of the antinutritional factors (NSP, phytate and oligosaccharides). Low moisture content in the desolventized meal (ie., 16-20%) resulted in no hydrolysis of phytate, oligosaccharide and NSP. However, the enzyme blend (blend III) pretreated canola meal based-diet significantly increased body weight gain and feed conversion ratio. The probable explanation for the improvement in broiler chicken performance when fed enzyme pretreated canola meal is that the enzymes were active in the GI tract.

A potential for improvement to the quality of canola meal by enzyme pretreatment is indicated by the results of this study, but more research is needed to implement the enzyme pretreatment technology on a commercial scale.

4. MANUSCRIPT II

THE APPETITE DEPRESSION MECHANISM IN CHICKEN FED PROTEASE SUPPLEMENTED DIETS

Abstract

Studies were conducted to investigate feed intake reduction observed in broiler chicken fed diets supplemented with exogenous protease compared to an unsupplemented control diet. The experimental diets included casein and sugar-based diets with or without protease supplementation. In order to explain the mode of action of protease enzymes, diets comprised of hydrolyzed casein and monosaccharides (glucose, fructose and galactose) were used. All diets were fed to 4-day old broiler chickens for a 2-week period. The digestibility (*in vitro*) of protein of the intact casein control diet supplemented with protease was about 17.8% compared to the control without enzyme and was 60% for the hydrolyzed casein diets. Both the protease supplemented diet and the hydrolyzed casein diet resulted in a significant reduction of feed intake and the hydrolyzed casein based diet reduced the feed conversion ratio ($P<0.05$). The hydrolyzed casein diets increased the jejunal amino acid digestibility ($P<0.05$) and free amino acids in plasma ($P<0.05$). The *in vitro* crop environment simulation study showed that protein digestibility was 18% and 60% for the protease supplemented and the hydrolyzed casein diets, respectively, which could result in high level of free amino acids in the crop. Therefore, the high level of free amino acids in the crop could cause a feed intake reduction by stimulation of the osmoreceptor. However, the significant reduction in feed intake with the hydrolyzed casein diet could be decreased further by the increased jejunal amino acid digestibility resulting in a high level of plasma free amino acids. An unbalanced amino acid absorption emphasizes the reduced feed intake and the high feed conversion ratio.

INTRODUCTION

The addition of enzymes to poultry diets is widely practiced. Over the last decade, knowledge has grown about the role enzymes can play in the digestion of feed ingredients (Annison, 1992). This will result in a more efficient use and an extended application of enzymes in poultry nutrition. In diets for young animals, in which the digestive system is not yet fully developed (Noy and Sklan, 1995), enzymes like amylases and proteases are used for better digestion of starch and proteins. The physical and chemical structures of the feed also limit the digestion in young animals. Provision of exogenous enzymes can supplement the endogenous enzyme activity of the bird, rendering certain nutrients more readily available for absorption, enhance the energy and nutrient value of lower grade raw materials and eliminate antinutritional factors.

Most studies show that enzyme (β-glucanase and xylanase) supplementation can decrease the viscosity and increase feed passage rate resulting in increase feed intake (White et al. 1981; Hesselman et al. 1982; Hesselman and Aman 1986; Petterson et al. 1990; Rotter et al. 1989). However, Simbaya et al. (1995) found that a substantial reduction in feed intake was observed in laying hens fed canola meal diets containing high level (0.5g kg^{-1}) of a protease supplement. Similar results have also been reported for broiler chickens by Sebastian et al. (1994) for a protease supplemented corn/soybean meal based diet. The reason is unclear. The objective of this study was to demonstrate the feed

intake depression with protease supplementation and to investigate the reason for the feed intake reduction with the use of protease enzyme supplementation.

MATERIALS AND METHOD

IN VITRO STUDY

In the standard assay procedure, 10g of diet was weighed into a 125ml Erlenmeyer flask and mixed with 80ml of distilled water. The samples were incubated at 40°C for 2hrs in an incubator-shaker (200rpm). The contents were transferred into pre-soaked dialysis tubes (Spectrum, Houston, TX, U.S.A.) with a molecular weight cut off value of 12000-14000 and the content of the flask were washed into the tube with 10ml of distilled water. The tubes were closed allowing for a small air gap in the tube to facilitate continued mixing of the contents (Kennedy et al., 1989). The tubes were attached to the metal frame mounted on a thermally controlled water bath (Bodine Electric Co., Chicago, Illinois, U.S.A.) with the metal frame rotating at approximately 20rpm. The dialysis continued for 72 h at 2°C with continuous rotation of the tubes against water. Dialysis water was changed at least twice a day. Following dialysis, the samples were transferred to plastic bags and frozen, freeze-dried and analyzed for amino acids. Samples of diets 1, 2, 3 and 6 (Table 21) were used in the *in vitro* study described above. The above method was used to mimic the crop environment and to estimate the rate of digestibility/solubility of protein in the crop. The amino acid digestibility/solubility was calculated using the following equation:

Table 21 Composition and calculated analysis of diets used in experiment (as fed basis)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredient (%)						
Sucrose	56.519	56.519	56.219	41.119	40.699	40.919
Fructose				7.7	7.16	7.65
Glucose				7.7	7.16	7.65
Galactose					1.5	
Casein (vitamin free)	20	20		20	20	
Casein (Hydrolyzed)			20.3			20.3
Gelatin	6	6	6	6	6	6
Oil (Vegetable)	3	3	3	3	3	3
Mineral premix[1]	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin premix[2]	2.2	2.2	2.2	2.2	2.2	2.2
Vitamin B58[3]	0.3	0.3	0.3	0.3	0.3	0.3
Calcium carbonate	2.3	2.3	2.3	2.3	2.3	2.3
$CaHPO_4$	1.2	1.2	1.2	1.2	1.2	1.2
KH_2PO_4	1.1	1.1	1.1	1.1	1.1	1.1
$NaHCO_3$	0.066	0.066	0.066	0.066	0.066	0.066
$MgSO_4.7H2O$	0.615	0.615	0.615	0.615	0.615	0.615
DL-Methionine	0.4	0.4	0.4	0.4	0.4	0.4
Arginine	0.25	0.25	0.25	0.25	0.25	0.25
Chromium Oxide	0.25	0.25	0.25	0.25	0.25	0.25
Alphacel	5	5	5	5	5	5
Enzyme[4]		0.025				
Total	100	100	100	100	100	100
Calculated composition(%)						
Protein	23.333	23.333	23.349	23.333	23.333	23.349
Metabolizable Energy (kcal/kg)	3293	3293	3298	3236	3234	3241
Calcium	1.14	1.14	1.14	1.14	1.14	1.14
AV. Phos	0.591	0.591	0.591	0.591	0.591	0.591

[1] Supplied (per kg of diet): Zn, 80mg; Mn, 88mg; Fe, 128mg; Cu, 8mg; Se, 0.16mg and I, 0.576.
[2] Supplied (per kg of diet): Vitamin A, 18150IU; Vitamin D_3, 2200IU; Vitamin E, 23.98IU; Vitamin B_{12},0.0253mg; Vitamin K, 2.42mg; Niacin 117.26mg; Riboflavin, 165mg; Choline chloride, 2244mg; Ca-Pantothenate, 24.2mg and Biotin; 0.55mg.
[3] Supplied (per kg of diet): Riboflavin, 13.2mg; Ca-Pantothenate, 26.4mg; Niacin, 39.6mg; Choline Chloride, 660mg.
[4] Supplied (per kg of diet): 0.025% of protease

$$\text{Amino acid digestibility/solubility (\%)} = \frac{AA_{diet} - AA_{residual}}{AA_{diet}} \times 100$$

AA_{diet}: amino acid content in diet
$AA_{residual}$: amino acid content in residual

IN VIVO STUDY

One day old chicks were obtained from a local commercial hatchery. From day 1 to 5, the birds were housed in Jamesway chick batteries and fed a commercial chick starter diet containing 20% protein. On day 5, birds were individually weighed and placed into narrow weight classes. Groups of five birds were then assigned to pens in Petersime brooder batteries such that all pens had a similar initial weight. Each treatment was randomly assigned to 7 replicates (pens) of five birds each. From Day 5 to 19, the birds were fed six experimental diets that were in a mash form (Table 21). A glucose-based purified diet containing intact casein as the main source of protein served as the control (diet 1). Diet 2 was the control diet supplemented with 0.025% protease enzyme (P-5147, Sigma-Aldrich). In diet 3 and 6 hydrolyzed casein was used instead of intact casein to test how quickly absorbed free amino acid influence feed intake. Diet 4, 5 and 6 monosaccharides replaced some of the sucrose to test if the feed intake reduction was caused by quick absorption of monosaccharides.

All birds had free access to water, feed and light throughout the experiments. Prior to initial, the 12- and 19-day weightings, the birds were fasted for 4h. Feed intake was recorded every other day for weeks 1 and 2 to calculate weekly and overall feed intakes

and feed conversion ratio. On day 15 of the experiment, 12 birds randomly selected from 7 replicates were killed by CO_2 asphyxiation. Ileal and jejunum contents of the birds were collected and were pooled for 3 birds (4 replicates/treatment), the samples were lyophilized and analyzed for amino acids. There were no differences with diet 3 and diet 4 on feed intake, body weight gain and feed conversion ratio relative to control diet, therefore, these groups were not used for digestion determinations.

On day 16 of the experiment, birds were fasted for 4 hours and then refed for 1 hour. Fifty birds were randomly selected from 7 replicates. Birds were killed by CO_2 asphyxiation and blood samples were taken by heart puncture using a syringe previously washed with a heparinized solution. The blood was then transferred to a heparinized tube and stored in an ice bath until it was centrifuged. The blood plasma was separated by centrifugation and plasma from 3 chicks was pooled (5 replicates/treatment). The samples of plasma were deproteinized by treating with sulphosalicylic acid (Angkanaporn, et. al., 1997), centrifuged and the supernatant was used to measure amino acid content. There were no difference with diet 3 and diet 4 on feed intake, body weight gain and feed conversion ratio relative to the control diet, therefore, these groups were not used for plasma amino acid determinations.

Pair Feeding

Based on a previous study in our laboratory (unpublished), chickens fed hydrolyzed casein diets reduce their feed intake. Pair feeding was included to account for the effect of feed intake on responses between the hydrolyzed casein group given free access to feed and

the restricted control groups (diet 1). This was accomplished by matching each pen of chicks fed the hydrolyzed casein diet with a pen which was fed the control diet once daily, at 1300h. The same amount of feed that the hydrolyzed casein fed chicks had eaten the previous day was supplied to the paired control groups.

CHEMICAL ANALYSIS

Diets, digesta (jejunum and ileum) and residual material after dialysis (*in vitro* experiment) were used for analysis of amino acids. All samples were subjected to 6N HCl and hydrolyzed for 24h at 110°C (Andrews and Baldar, 1985). Acid hydrolysates were subsequently analyzed for amino acid content by ion exchange chromatography (LKB Biochrom 4151, Alphaplus amino acid analyzer, Biochrom, Science park, Cambridge, UK.). Analysis of methionine and cystine were conducted using performic acid oxidation by the method of Moore (1963). The plasma free amino acids were determined using the method described by Hubbard et. al. (1988).

STATISTICAL ANALYSIS

Data were subjected to ANOVA for completely randomized designs using SAS® (SAS Institute, 1990). Statistical significance of differences among treatments was assessed using the Duncan's difference test (Duncan, 1955).

RESULTS AND DISCUSSION

In vitro study

The results of the digestibility/solubility of protein following *in vitro* incubation and dialysis are shown in Table 22. The average amino acid digestibility/solubility was zero for diet 1 (control). However, the digestibility of diet 2 (with enzyme) is about 18%. Since diets 3 and 6 used hydrolyzed casein (high level of free amino acid), the amino acid digestibility was relatively high at approximately 60%.

Since the *in vitro* study was to mimic the environment of the crop, it could be suggested that protein can be digested and cause a high level of free amino acids in the crop with protease supplementation. Therefore, the high level of free amino acid in the crop would reduce feed intake by stimulation of osmoreceptors. Shurlock and Forbes (1981) reported that the avian crop contained areas that play a part in food intake regulation. They found that infusion of glucose and non-nutritive hypertonic solutions into the crop significantly reduced feed intake, and suggested that the crop infusion reduced feed intake by the stimulation of osmorecepters.

In vivo study

Birds fed the hydrolyzed casein had a significantly ($P<0.05$) lower feed intake than those fed the intact casein diet in the first 10 days (Table 23), but as adaptation in amino acid catabolism occurs, the birds were able to restore feed consumption by day 12. There were no differences of feed intake for diets 4 and 5 relative to diet 1 (control). This may suggest

Table 22. The digestibility/solubility of amino acid *in vitro* study (%)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 6
ASP	0.49±3.77[1]	15.80±0.66	49.72±0.78	48.60±0.19
THR	-0.60±3.97	13.33±1.08	52.54±0.52	58.83±1.46
SER	-4.12±4.97	11.13±0.93	45.20±0.29	47.94±0.78
GLU	-1.00±4.74	15.28±0.09	55.39±0.24	56.37±0.35
PRO	-6.01±13.36	17.22±0.53	50.64±1.57	51.84±0.83
GLY	10.25±3.16	42.35±0.96	28.02±0.34	24.45±1.30
ALA	3.24±3.32	27.35±0.14	44.49±5.06	41.37±0.10
VAL	-13.19±10.99	10.81±6.26	60.01±1.36	58.29±1.15
ILE	-9.82±12.86	14.15±2.62	49.00±3.66	54.37±0.24
LEU	-7.29±8.66	11.85±0.50	71.35±0.67	71.34±1.71
TYR	-34.78±11.24	-22.72±2.19	100.00±0	100.00±0
PHE	-3.76±9.41	15.84±0.59	80.36±0.33	79.35±0.48
HIS	-6.04±12.14	12.61±0.72	65.65±3.89	64.54±0.18
LYS	-5.44±10.03	12.40±0.50	76.72±2.66	77.34±0.52
ARG	16.27±9.97	43.91±0.25	68.55±0.89	64.64±0.31
CYS	5.45±0.89	18.65±10.63	42.38±1.43	47.48±1.83
MET	42.23±0.06	49.47±0.46	85.02±0.35	85.32±2.01
AVE	**-0.86±7.16**	**18.20±1.14**	**60.30±0.10**	**60.71±0.62**

[1]Value±SD

Table 23. Daily feed intake (g/bird)

Diet	0 - 2	2 - 4	4 - 6	6 - 8	8 - 10	10 - 12
1	20.81[a1]	28.72[a]	35.13[a]	40.58[a]	50.90[a]	47.83[a]
2	19.65[b]	26.93[a]	32.46[a]	36.37[bc]	44.73[b]	46.21[a]
3	15.59[c]	23.04[b]	26.06[c]	33.35[c]	37.87[c]	46.10[a]
4	20.47[ab]	28.57[a]	32.93[a]	39.10[ab]	49.41[a]	50.22[a]
5	20.91[a]	28.76[a]	33.14[a]	39.76[a]	48.45[ab]	50.28[a]
6	16.22[c]	24.24[b]	29.20[b]	35.46[c]	37.12[c]	47.22[a]
SEM	0.56	0.85	1.41	1.56	2.05	2.37

[1] Means within a column with no common superscript differ (P<0.05)

that sucrose digestion and absorption was similar to the monosaccharides. Feed intake of the enzyme supplemented diet was significantly ($P \leq 0.05$) lower than the control diet on day 2, 8 and 10.

The results of this experiment supports previous observation (Simbaya et al., 1995; Sebastian et al., 1994) that protease supplemented diets reduced overall feed intake. To explain the mode of action of protease enzymes, hydrolyzed casein and simple sugar were used in purified diets. It should be pointed out that intake of the simple sugar supplemented diets were not different from the control diet. However, the feed intake of birds fed the hydrolyzed casein diets was greatly reduced, more so than for the protease supplemented diet.

The overall results of the 2-week performance trial of broiler chicks are given in Table 24. The protease supplemented diet resulted in a significant decrease in feed intake and body weight gain as compared to control diet. The chicks fed the hydrolyzed casein diet (diet 3 and diet 6) had a significantly ($P<0.05$) lower feed intake and weight gain than the control diet, and the feed conversion ratio was also significantly ($P<0.05$) poorer. However, the pair fed control had significantly higher weight gain and a better feed conversion ratio than those fed diets 3 and 6, and there was no significant difference in feed conversion between the pair fed control groups and control diet. Feed intake, body weight gain and feed conversion ratio were similar for birds fed diets 4 and 5 and diet 1 (control).

Table 24. The overall feed intake, feed gain and feed efficiency

Diet	Feed Intake (g/ bird)	Body Gain (g/ bird)	Feed conversion Ratio
1	564.1[a1]	442.6[a]	1.28[cd]
2	516.1[b]	418.5[b]	1.23[d]
3[2]	461.5[c]	256.6[e]	1.80[a]
4	558.2[a]	438.0[ab]	1.27[cd]
5	562.0[a]	445.6[a]	1.27[d]
6	478.0[c]	285.7[d]	1.68[b]
Pair fed group	430.1[d]	323.5[c]	1.33[c]
SEM	12.8	10.0	0.03

[1] Means within a column with no common superscript differ ($P<0.05$)
[2] Paired fed diet

Jejunal and ileal amino acid digestibility and plasma free amino acids

The amino acid digestibilities calculated from jejunal digesta of birds fed the experimental diets are shown in Table 25. The jejunal amino acid digestibility of birds fed hydrolysed casein diets were higher ($P<0.05$) than the control diet with essential amino acids. There were no differences for the jejunum amino acid digestibility between the protease supplemented diet (diet 2) and control diet (diets). However, all the treatment differences, except for histidine and arginine, had disappeared when the digesta reached the ileal compartment of the digestive tract (Table 26).

The plasma concentrations of free amino acids are shown in Table 27. As expected, plasma concentrations of most essential amino acids and the total content were higher in birds fed hydrolyzed casein diets than the control. The average free amino acids in diet 3 and diet 6 were 158mg/dl and 163mg/dl, which were 46% and 51%, respectively, higher than for the control diet. Protease supplementation did not influence plasma free amino acids.

The increase in amino acid digestibility at the jejunum with diets 3 and 6 can partially explain the feed intake reduction, since it coincided with the high plasma concentration of free amino acids for the hydrolyzed casein diets. It has been known for many years that plasma amino acids affect feed intake. Mellinkoff et al. (1956) observed that the serum amino acid concentration correlated inversely with appetite in man.

The result also showed that the increase of essential amino acids in the plasma was not uniform. Threonine for instance was the lowest with a 21% (diet 3) and 18% (diet 6)

Table 25. The amino acid digestibility calculated from jejunal digesta (% dry matter)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 6	SME
ASP	75.8	79.2	85.4	84.9	3.2
THR[1]	**70.3[b2]**	**75.4[ab]**	**84.2[a]**	**83.4[a]**	**4.2**
SER	69.7	75.1	82.7	81.9	3.7
GLU	72.5	76.8	88.9	87.6	7.6
PRO	74.4	79.5	85.3	83.4	3.2
GLY	75.1	73.6	80.5	77.4	4.8
ALA	80.2	82.7	87.7	85.1	2.7
VAL	**73.5[b]**	**79.1[ab]**	**88.1[a]**	**86.9[a]**	**4.2**
ILE	**72[b]**	**78[b]**	**87[a]**	**87[a]**	**4.2**
LEU	**81.1[c]**	**84.2[bc]**	**93.0[a]**	**91.9[ab]**	**3.8**
TYR	76.6	80.0	94.1	93.2	5.0
PHE	**80.8[b]**	**84.1[b]**	**94.2[a]**	**92.9[a]**	**3.9**
HIS	**75.8[b]**	**79.8[ab]**	**86.6[a]**	**85.1[a]**	**3.7**
LYS	**81.4[b]**	**84.2[b]**	**93.6[a]**	**92.6[a]**	**3.6**
ARG	**85.8[b]**	**87.9[ab]**	**93.9[a]**	**92.1[a]**	**2.7**
CYS	61.9	67.7	76.8	77.0	4.2
MET	**91.5[c]**	**92.3[bc]**	**96.4[ab]**	**95.6[a]**	**1.7**
Average	**76.4[b]**	**80.0[ab]**	**88.2[a]**	**86.9[a]**	**3.5**

[1]Number with bold is essential amino acid for chick

[2]Means within a row with no common superscript differ (P<0.05)

Table 26. The amino acid digestibility calculated from ileal digesta (% dry matter)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 6	SME
ASP	91.1	90.4	89.9	89.3	1.0
THR[1]	**89.0**[a2]	**89.2**[a]	**89.7**[a]	**89.3**[a]	**1.0**
SER	87.4	87.5	86.9	86.8	2.1
GLU	93.3	93.5	91.3	91.5	1.0
PRO	91.6	91.7	90.2	89.3	1.4
GLY	88.9	87.0	87.6	86.2	2.0
ALA	93.2	93.1	92.7	91.5	1.0
VAL	**91.9**[a]	**93.2**[a]	**93.0**[a]	**91.6**[a]	**1.0**
ILE	**91.3**[a]	**92.5**[a]	**92.4**[a]	**91.4**[a]	**1.4**
LEU	**96.3**[a]	**96.7**[a]	**96.3**[a]	**95.8**[a]	**0.6**
TYR	96.5	96.9	96.7	96.5	0.7
PHE	**97.1**[a]	**97.7**[a]	**97.6**[a]	**97.2**[a]	**0.4**
HIS	**92.9**[a]	**93.1**[a]	**90.6**[b]	**89.4**[b]	**0.7**
LYS	**96.6**[ab]	**97.1**[a]	**96.2**[ab]	**95.9**[b]	**0.5**
ARG	**97.4**[a]	**97.8**[a]	**96.9**[a]	**96.0**[b]	**0.4**
CYS	77.5	76.3	76.3	82.5	4.9
MET	**97.8**[a]	**97.7**[a]	**97.4**[a]	**97.5**[a]	**0.4**
Average	**92.3**[a]	**92.4**[a]	**91.9**[a]	**91.6**[a]	**1.0**

[1] Number with bold is essential amino acid for chick

[2] Means within a row with no common superscript differ (P<0.05)

Table 27. The free amino acid profile of plasma (μg/ml)

Amino acid	Diet 1	Diet 2	Diet 3	Diet 6	SME
ASPARTIC ACID	8.6	7.8	12.7	12.7	1.0
HYDROXYPROLINE	57.8	61.5	66.4	69.2	7.8
THREONINE[1]	**108.2**[bc2]	**94.7**[c]	**137.1**[a]	**131.2**[ab]	**11.0**
SERINE	108	91.7	119.9	124.5	13.1
ASPARAGINE	43.9	43.9	92.6	100.8	6.7
GLUTAMIC ACID	56.8	59.7	83.8	86	7.4
GLUTAMINE	94	106.9	159.7	166.5	15.0
SARCOSINE	6.8	6.4	17.3	13.4	3.5
PROLINE	89.5	88.2	159.2	159.3	9.8
GLYCINE	84.6	70.8	83.1	84.1	8.7
ALANINE	66.3	65.5	94	94.1	4.9
CITRULLINE	1.1	1.2	2.7	2.1	0.6
VALINE	**43.2**[b]	**40.2**[b]	**79.4**[a]	**82.1**[a]	**4.1**
CYSTINE	12.7	11.6	14.5	14.8	1.1
METHIONINE	**36.1**[b]	**30.1**[b]	**50.9**[a]	**55.1**[a]	**4.6**
L-CYSTATHIONINE	8.7	9.2	10.3	10	0.9
ISOLEUCINE	**15.3**[b]	**13.2**[b]	**29.7**[a]	**32.6**[a]	**1.4**
LEUCINE	**27.0**[b]	**22.7**[b]	**44.3**[a]	**47.3**[a]	**2.5**
TYROSINE	28.5	27.6	58.7	66.6	5.7
B-ALANINE	1.3	0.6	2.4	1.7	0.1
PHENYLALANINE	**19.1**[b]	**15.8**[b]	**28.3**[a]	**30.9**[a]	**1.8**
HYDROXYLYSINE	1.8	1.4	2.2	2.1	0.6
ORNITHINE	5.4	3.2	8.8	8.1	1.4
LYSINE	**90.9**[b]	**84.1**[b]	**128.1**[a]	**131.8**[a]	**10.2**
HISTIDINE	**15.2**[b]	**11.9**[b]	**26.8**[a]	**28.8**[a]	**2.2**
TRYPTOPHAN	**5.3**[b]	**5.1**[b]	**9.2**[ab]	**10.5**[a]	**2.0**
ANSERINE	3.3	2	4.7	5.8	0.9
ARGININE	**45.7**[ab]	**36.0**[b]	**55.0**[a]	**56.8**[a]	**6.9**
Total	**1084.9**[b]	**1013.2**[b]	**1581.8**[a]	**1628.8**[a]	**91**

[1]Number with bold is essential amino acid for chick

[2]Means within a row with no common superscript differ (P<0.05)

increase relatively to diet 1 (control), whereas isoleucine was the highest with 94% (diet 3) and 113% (diet 6) increase compared to the control diet (Table 27).

The uneven increase would result in an imbalance in the amino acid profile. It has been known for years that the balance of amino acids markedly influences food intake in both chicks and rats. Unbalanced diets cause rapid decrease in food intake and altered patterns of feeding and the overall efficiency of utilization of dietary protein (Rogers and Leung, 1973; Kumta et al., 1958). Salmon (1954) suggested that surplus amino acids causing an imbalance might stimulate catabolic pathways resulting in the degradation of all amino acids, with the inadvertent loss of the limiting amino acids. It is known that consumption of an unbalanced diet will cause a deranged pattern of plasma amino acids to reach the brain, thus impairing the synthesis of proteins used in neural pathways, which in turn may lead to a reduction of feed intake (Forbes 1995, Harper et al, 1970).

Several studies with pigs indicate that amino acid imbalances may occur at the tissue level even though the diet may appear to be in ideal balance. Such imbalances are readily demonstrated on supplementation of cereal-based diets with crystalline amino acids. It has long been recognized that free amino acid supplements are absorbed more rapidly than protein-bound amino acids resulting in an imbalance supply of amino acids at the sites of protein synthesis (Rolls et al., 1972; Leibholz et al., 1986; Leibholz, 1989). Leibholz et al. (1986) observed that the concentration of free lysine in plasma of pigs increased 1-2 hours after feeding a diet containing crystalline lysine, declining thereafter, whereas the circulating concentrations of other amino acids derived from the protein-bound fraction of the diet peaked 2-6 hours after feeding. In pigs fed once daily, this lack of synchrony in absorption

would precipitate an amino acid imbalance at the cellular level. Under these circumstances growth and efficiency of utilization of dietary nitrogen (N) may be impaired. The current study concurred with this report. However, even though the hydrolyzed casein based diets were in ideal balance according to NRC (1994), the high level of free amino acid could be quickly absorbed which caused an unbalanced supply of amino acids to the chickens. It is unclear why the protease supplemented diet did not change the plasma free amino acid profile and yet significantly reduced feed intake.

Sanahuja and Harper (1962) reported that when unbalanced diets are fed, rats quickly develop a strong aversion to the diet, although food intake slowly increases with prolonged exposure to the diet. Similar results were found in the current study in that feed intake depression recovered by the end of two week feeding of the hydrolyzed casein and protease supplemented diets

CONCLUSION

Feeding diets containing a protease enzyme or hydrolyzed casein depressed feed intake and reduced weight gains in chickens. The hydrolyzed casein diets had a more deleterious effect on feed intake and growth than the protease supplemented diet.

The hydrolyzed casein diet increased jejunal amino acid digestibility, but not ileal amino acid digestibility. Unlike hydrolyzed casein diets, the protease supplemented diet did not affect the plasma free amino acid concentration.

The mechanism of feed intake depression caused by the protease supplemented diet or hydrolyzed casein diets may be due to increased concentration of free amino acid in the crop that cause feed intake reduction by stimulation of osmoreceptor.

The reduction in feed intake with the hydrolyzed casein diet could be decreased further by the increasing jejunal amino acid digestibility and a high level of plasma free amino acid, and an unbalanced amino acid absorption which may emphasized the reduced feed intake and high feed conversion ratio.

The availability of monosaccharides verses sucrose, had a no effect on feed intake in this study.

5. GENERAL DISCUSSION

Exogenous enzymes are widely used as feed supplement to eliminate the anitnutritional factors in cereal based diets. Supplementation of exogenous enzyme to those diets can enhance feed digestibility, eliminate anti-nutritional factors, improve feed conversion ratio, thereby reducing feed cost while maintaining bird performance (Bedford and Morgan, 1996). With canola meal, some reports have also indicated that supplementation with exogenous enzymes can improve fibre digestibility (Slominski and Campbell, 1990; Alloui et al., 1994; Slominski et al., 1997) and improve bird performance (Slominski et al., 1997). However, there has been relatively little research conducted on the use of enzyme pretreatment method to improve the quality of canola meal and how protease enzymes influence feed intake. These experiments were conducted to investigate the potential for improving the nutritive value of the meal by using an enzyme pretreatment method to reduce the negative effects of fibre and phytate. Twelve commercial carbohydrase-like (ie., α-galactosidase, pectinase) and two phytase enzymes were evaluated *in vitro* to select the best enzyme blend. Results showed that incubation of canola meal (80% moisture) with enzymes resulted in varying degrees of NSP or phytate hydrolysis. Carbohydrases and phytases were selected on the basis of their ability to reduce NSP and phytate, their specific enzyme activity and source (manufacturer).

When enzyme blends were used, it had an additive effect on NSP (blend III) and phytate (blend I, II and III) reduction *in vitro*. In general, findings from the current study were in

agreement with previous studies from this laboratory (Slominski et al., 1999). Slominski et al (1999) reported that *in vitro* canola meal with exogenous enzyme blends (carbohydrases and phytase) can partially of depolymerize the cell wall polysaccharide, increase the soluble NSP, and reduce the phytate content.

Results with enzyme blends (blend I, II, III and IV) pretreated canola meal at industrial condition (16 – 20% moisture content in desolventized meal) showed that no hydrolysis of NSP, oligosaccharide or phytate occurred. Possibly this is due to the low moisture content. Slominski et al. (1999) reported that the efficacy of enzyme pretreatment was greatly influenced by moisture content, with 70 – 80% showing the highest degree of NSP and phytate hydrolysis. At 40% moisture content there was a little reduction in total NSP and phytate content. However, when enzyme pretreated canola meal based diets were fed to 4-day old broiler chickens for a 2-week period, there was improved growth performance and feed conversion ratio with enzyme blend III relative to the control diet. The *in vivo* improvement in performance with enzyme pretreated canola meal probably was due to enzyme activity occurring in the GI tract of the broiler chicken. Another explanation for the improvement of bird performance was the fact that exogenous enzyme can destroy the cell wall matrix and release the cell content that would be easy to digest in small intestine (Campbell and van der Poel, 1998).

There are few studies in the literature that evaluated the enzyme effect on ileal protein digestibility, AMEn content, total tract digestibility of phytate, NSP and oligosaccharides. The results of the current studies showed that protein and phytate digestibility of pretreated canola meal were significantly ($P<0.05$) improved by three enzyme blends (blend I, II and

III), but only enzyme blend II and III showed a significant ($P<0.05$) AMEn improvement. Slominski et al. (1999) reported that the enzyme pretreated canola meal incorporated into a semi-purified diet at the 30% level in a rat growth trial improved the total dietary energy, phytate and available phosphorus contents.

Since canola and soybean meal, are protein supplements in poultry diets, it would be very important to improve their protein digestibility. Protease, besides carbohydrase and phytase, could be selected because it would increase protein digestibility directly. However, studies with protease supplementation have shown a in decrease feed intake of chicken fed in canola meal (Simbaya et al. 1996) and soybean meal (Sebastian et al. 1994), but the reason is unknown.

The current research with protease supplementation of semi-purified diets was conducted to investigate the reason of this effect. The results of this study indicated that protease supplementation of a casein based diet and the hydrolyzed casein based diet depressed feed intake. Based on the *in vitro* study that was conducted to mimic the crop environment, it was suggested that there was a substantial release of free amino acids from the protease supplemented and hydrolyzed casein diet. The high levels of free amino acids in the crop may cause feed intake depression via their effect on the osmoreceptor. This can explain why the protease supplemented diet reduced feed intake. The reason for further feed intake reduction with the hydrolyzed casein diet may be due to the increased jejunal digestibility of most essential amino acids, which might result in a surplus supply of amino acids in the gastro-intestinal tract. The surplus supply of amino acids could result in high

level of free amino acids in plasma that would further reduce feed intake of chickens (Denbow, 1994).

6. CONCLUSION

1. *In vitro* enzyme treatment of canola meal under 80% moisture conditions was found to effectively reduce most of the antinutritional factors (NSP, phytate and oligosaccharids).
2. Low moisture content in the desolventized meal (ie., 16-20%) resulted in no hydrolysis of phytate, oligosaccharide and NSP from enzyme treatment.
3. The enzyme blend (blend III) pretreated canola meal based diet significantly increased body weight gain and feed conversion ratio. The probable explanation for the improvement in broiler chicken performance when fed enzyme pretreated canola meal is that the enzymes were being active in the GI tract.
4. A potential for improvement to the quality of canola meal by enzyme pretreatment is indicated by the results of this study, but more research is needed to implement the enzyme treatment on a commercial basis.
5. Both the protease supplemented and hydrolyzed caseins diet depressed feed intake and body weight gain.
6. Hydrolyzed casein diet increased jejunal protein digestibility, all diets had similar ileal digestibility.
7. The hydrolyzed casein diets increased the free amino acid level of plasma, but this was not the case for the protease supplemented diet.
8. The mechanism of feed intake depression caused by the protease supplemented diet and hydrolyzed casein diets may be due to increased concentration of free amino acids in the crop that cause feed intake reduction by stimulation of osmorecepters.

9. The reduction in feed intake with the hydrolyzed casein diet could further be decreased by the increasing jejunal amino acid digestibility and a high level of plasma free amino acids, as well as an unbalanced amino acid absorption.

7. REFERENCES

Alloui, O., M. Chibowska, and S. Smulikowska, 1994. Effects of enzyme supplementation on the digestion of low glucosinolate rapeseed meal *in vitro*, and its utilisation by broiler chicks. J. Anim. Feed Sci. 3:119-128

Almquist, H. J., 1954. Utilisation of amino acids by chicks. Arch. Biochem. Biophys. 52: 197-201

Anderson-Hafermann, J. C., Y. Zhang, and C. M. Parsons, 1993. Effects of processing on the nutritional quality of canola meal. Poultry Sci. 72:326-333

Andrews, R. P. and N. A. Baldar, 1985. Amino acid analysis of feed constituents. Science Tools. 32: 44-48

Angkanaporn, R., V. Ravindran, Y. Mollan, and W. L. Bryden, 1997. Homoarginine influences voluntary feed intake, tissue basic amino acid concentrations and arginase activity in chickens. J. Nutr. 127:1128-1136

Annison, G., and M. Choct, 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. World's Poultry Sci. J. 47:232-242

Annison, G., 1992. Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolizable energy, starch and pentosan digestibilities in broiler chickens. Anim. Feed Sci. Technol. 38:105-121

Antoniou, T. C., R. R. Marquardt, and E. Cansfield, 1981. Isolation, partial characterization, and antinutritional activity of a factor (pentosans) in rye grain. J. Agr. Food Chem. 29:1240-1247

Antoniou, T. C., and R. R. Marquardt, 1982. The utilization of rye by growing chicks as influenced by autoclave, treatment, water extraction and water soaking. Poultry Sci. 61:91-102

Association of Official Analytical Chemists. 1990. Official methods of analysis Helrich, K., (editor). AOAC Inc. Arlington. VA. U.S.A.

Barbour, G. W., and J. S. Sim, 1991. True metabolizable energy and true amino acids availability in canola and flax products for poultry. Poultry Sci. 70:2154-2160

Bedford, M. R., and H. L. Classen, 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. J. Nutr. 122:560-569

Bedford, M. R.,1995. Mechanism of action and potential environmental benefits from the use of feed enzymes, Anim. Feed. Sci. Technol. 53:145-155

Bedford, M. R., and A. J. Morgan, 1996. The use of enzymes in poultry diets. World's Poultry Sci. J. 52:61-68

Bedford, M. R., and H. Schulze, 1998. Exogenous enzymes for pigs and poultry. Nutrition Res. Reviews 11:91-114

Bell, J. M., and M. O. Keith, 1991. A survey of variation in the chemical composition of commercial canola meal produced in Western Canadian crushing plants. Can. J. Anim. Sci. 71:469-480

Bell, J. M., M. O. Keith, and D. S. Hutcheson, 1991. Nutritional evaluate of very low glucosinolate canola meal. Can. J. Anim. Sci. 71:497-506

Bell, J. M., 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73:679-697

Bjergegaard, C., B. O. Eggum, S. K. Jensen, and H. Sørensen, 1991. Dietary fiber in oilseed rape: physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diets. J. Anim. Physiol. and Nutr. 66:69-79

Bjergegaard, C., S. K. Jensen, A. Quinsac, and H. Sørensen, 1998. Analyses of antinutritional factors in rapeseed, in Recent Advances of Research in Antinutritional Factor in Legume Seeds and Rapeseed. Wageningen, The Netherlands, 8-10 July 1998. EAAP Publication No. 93

Blair, R., and R. D. Reichert, 1984. Carbohydrate and phenolic constituents in a comprehensive range of rapeseed and canola fractions: Nutritional significance for animals. J. Sci. Food Agric. 35:29-35

Burnett, G. S., 1962. The effect of damaged starch, amylolytic enzymes and proteolytic enzymes on the utilization of cereals by chickens. Br. Poultry Sci. 3:89-103

Butler, E. J., A. W. Pearson, and G. R. Fenwick, 1982. Problems which limit the use of rapeseed meal as a protein source in poultry diets. J. Sci. Food Agric. 33:866-875

Campbell, G. L., and A. F. B. van der Poel, 1998. Use of enzymes and process technology to inactivate antinutritional factors in legume seeds and rapeseed in Antinutritional Factor in Legume Seeds and Rapeseed. Wageningen, The Netherlands, 8-10 July 1998. EAAP Publication No. 93

Campbell, L. D., and F. Schöne, 1998. Effects of antinutritional factors in rapeseed in Antinutritional Factor in Legume Seeds and Rapeseed. Wageningen, The Netherlands, 8-10 July 1998. EAAP Publication No. 93

Canola Council of Canada, 1997. Canola meal feed industry guide. Hickling D. (editor) Canola Council of Canada, Winnipeg, MB

Carnovale, E., E. Lugaro, and G. Lombardi-Boccia, 1988. Phytic acid in Faba bean and pea: Effect on protein availability. Cereal Chem. 65:114-117

Cheryan, M., 1980. Phytic acid interactions in food systems. CRC. Critical Review in Food Science and Nutrition 13: 297-335

Chesson, A., 1987. Supplementary enzymes to improve the utilization of pig and poultry diets. In: Recent Advances in Animal Nutrition (Eds Haresign, W. and Cole, D. J. A.), Butterworths; London, pp. 71-89

Chesson, A., 1993. Feed enzymes, Anim. Feed. Sci. Technol.,45:65-79

Choct, M., and G. Annison, 1992. The inhabitation of nutrient digestion by wheat pentosans. Br. J. Nutr. 67:123-132

Choct, M., R. J. Hughes, R. P. Trimble, K. Angkanaporn, and G. Annison, 1995. Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat and low apparent metabolizable energy. J. Nutr. 125:485-492

Clandinin, D. R., and A. R. Robblee, 1983. Apparent and true metabolizable energy values for low glucosinolate-type rapeseed meal. Feedstuffs Vol. 55: pp.5 and 20

Clandinin, D. R., ed., 1989. Canola Meal for Livestock and Poultry. Canola Council of Canada, Winnipeg, MB, Canada.

Classen, H. L., J. M. Bell, and W. D. Clark, 1991. Nutritional value of very low glucosinolate canola meal for broiler chickens. pp 390-395 in Proceeding 8th Int. Rapeseed. Saskatoon, Saskatchewan, Canada.

Coon, C. N., K. L. Leske, O. Akavanichan, and T. K. Cheng, 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fibre digestion in adult roosters. Poultry Sci. 69:787-793

Cowan, W. D., A. Korsbak, T. Hastrup, and P. B. Rasmussen, 1996. Influence of added microbial enzymes on energy and protein availability of selected feed ingredients. Anim. Feed Sci. Technol. 60: 311-319

Dale, N., 1996. Variation in feed ingredient quality: oilseed meals. Anim. Feed Sci. Technol. 59:129-135

de Lange, C. F. M., W. B. Souffrant, and W. C. Sauer, 1990. Real ileal protein and amino acid digestibilities in feedstuffs for growing pigs as determined with the ^{15}N-isotope dilution technique. J. Anim. Sci. 68:409-418

de Rharn, O., and T. Jost, 1979. Phytate-protein interactions in soybean extracts and low- phytate soy protein products. Journal of Food Science 44: 595-601

Denbow, D. M., V. Ravindran, E. T. Kornegay, Z. Yi, and R. M. Hulet, 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poultry Sci. 74:1831-1842

Denbow, D. M., 1994. Appetite and its control. Poultry Sci. 5: 209-229

Duncan, D. G., 1955. Multiple range and multiple F-test. Biometrica 11:1-42

Edwards, H. M. Jr., 1983. Phorphorus. 1. Effect of breed and strain on utilization of sub-optimal levels of phosphorus in the ration. Poultry Sci. 62:77-84

Fenwick, G. R., and R. F. Curtis, 1980. Rapeseed meal and its use in poultry diets. a review. Anim. Feed Sci. Technol. 5:255-298

Ferket, P. R., 1993. Practical use of feed enzymes for turkeys and broilers. J. Appl. Poultry Res. 2:75-81

Finlayson, A. J., 1974. The amino acid composition of rapeseed hulls. Can. J. Anim. Sci. 54: 495-496

Forbes, J. M. 1995. Volumtary food intake and diet selection in farm animals. CAB International Wallingford, U. K.

Friesen, O. D., W. Guenter, R. R. Marquardt, and B. A. Rotter, 1992. The effect of enzyme supplementation of the apparent metabolizable energy and nutrient digestibilities of wheat, barley, oats, and rye for young broiler chicks. Poultry Sci. 71:1710-1721

Gauthier, S., G. Vachon, J. D. Jones, and L. Savoie, 1982. Assessment of protein digestibility by *in vitro* enzymatic hydrolysis with simultaneous dialysis, J. Nutr. 112:1718-1722

Guenter, W., B. A. Slominski, J. Simbaya, and L. D. Campbell, 1995. Potential for improved utilization of canola meal using exogenous enzymes. In: Proceedings of the 9th Internation Rapeseed Congress, Cambridge, U. K. pp 164-166

Guenter, W., 1997. Phytases in cereals and hemicelluloses in canola (rapeseed) meal and lupins. In: Enzymes in Poultry and Swine Nutrition. Ed. R.R. Marquardt and Z. Han, IDRC.pp.99-114.

Hancock, J. D., E. R. Peo, Jr., A. J. Lewis, and J. D. Crenshaw, 1990. Effects of ethanol extraction and duration of heat treatment of soybean flakes on the utilization of soybean protein by growing rats and pids. J. Anim. Sci. 68:3233-3243

Harper, A. E., N. J. Benevenga, and R. M. Wohlhueter, 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50: 428-558

Hesselman, K. and P. Aman, 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low- or high-viscosity. Feed Sci. Technol. 15:83-93

Hesselman, K., K. Elwinger, and S. Thomke, 1982. Influence of increasing levels of β-glucanase on the productive value of barley diets for broiler chickens. Anim. Feed Sci. Technol. 7:351-358

Hill, R. W., D. L. Anderson, R. Renner, and L. G. Jr. Carew, 1960. Studies on the metabolizable energy of grain and grain products for chickens. Poultry Sci. 39:573-579

Hotten, P., 1991. Why consider enzymes as a feed additive? Misset-World Poult.7:13-15

Hubbard, R. W., J. G., Chambers, and A. Sanchez, 1988. Amino acid analysis of plasma: Studies in sample preparation. J. Chromatog. 431:163-169

Imbeah, M., and W. C. Sauer, 1991. The effect of dietary level of fat on amino acid digestibilities in soybean meal and canola meal and on rate of passage in growing pigs. Livestock Production Science. 29:227-239

Jackson, M., 1998. Comparison of 5 commercial enzymes in corn-soybean meal diets on broiler chick performance. Poultry Sci. (Supple. 1) 62: 55

Jensen, L. S., R. E. Fru, J. G. Allred, and J. McGinnis, 1957. Improvement in the nutritional value of barley for hicks by enzyme supplementation. Poultry Sci. 36:919-921

Jensen, S. K., Y. G. Liu, and B. O. Eggum, 1995. The influence of feed size and hull content on the composition and digestibility of rapeseed in rats. Anim. Feed Sci. Technol. 54: 9-19

Jensen, S. K., 1999. Improvement of the nutritive value of rapeseed by selecting varieties with very low glucosinolate content. In proceeding of the 10th international rapeseed congress, Sept. 26-29, 1999, Canberra, Australia.

Kennedy, J. F., R. J. Noy, J. A. Stead, and C. A. White, 1989. A new method for predicting *in vitro* protein quality (PDD index). Food Chem. 32:277-295

Kornegay, E. T., D. M. Denbow, Z. Yi, and V. Ravindran, 1996. Response of broilers to graded levels of microbial phytase added to maize-soybean-meal diets containing three levels of nonphytate phosphorus. Br. J. Nutr. 66:251-259

Kumta. U. S., A. E. Harper, and C. A. Elvehjem, 1958. Amino acid imbalance and nitrogen retention on adult rats. J. Biol. Chem 233: 1508

Lee, K. H., G. H. Qi, and J. S. Sim, 1995. Metabolizable energy and amino acid availability of full-fat seeds, meals, and oils of flax and canola. Poultry Sci. 74:1341-1348

Leibholz, J., R. J. Love, Y. Mollah, and R. R. Carter, 1986. The absorption of dietary L-lysine and extruded L-lysine in pigs. Anim. Feed Sci. Technol. 15: 141-148

Leibholz, J. 1989. The utilisation of the free and protein-bound lysine. In Friedman, M. (ed) Absorption and utilisation of amino aicds, Vol. III. CRC Press, Boca Ration, PP. 175-187

Leslie, A. J., J. D. Summers, and J. D. Jones, 1973. Nutritive value of air-classified rapeseed fractions. Can. J. Anim. Sci. 53:153-156

Lessire, J., J. J. Baudet, J. M. Hallouis, and L. Conan, 1991. Metabolizable energy content and protein digestibility of rapeseed hulls in adult cockerels. pp 1585-1590 in Proceeding 8th Int. Rapeseed. Saskatoon, Saskatchewan, Canada.

Li, E. T. S. and G. H. Anderson, 1983. Amino acids in the regulation of food intake. Nutrition abstracts and reviews 53:169-181

Liu, Y. G. S. K. Jensen, and B. O. Eggum, 1995. The influence of seed size on digestibility and growth performance of broiler chickens fed full-fat rapeseed. J. Sci. Food Agric. 67:135-140

Lodhi, G. N., R. Renner, and D. R. Clandinin, 1969. Available carbohydrate in rapeseed meal and soybean meal as determined by a chemical mehod and a chick bioassay. J. Nutr. 99:413-418

Marquardt, R. R., D. Boros, W. Guenter, and G. Crow, 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by the use of a *Trichoderma reesei* enzyme preparation. Anim. Feed Sci. Technol. 45:363

Marquardt, R. R., A. Brenes, Z. Zhang, and D. Boros, 1996. Use of enzymes to improve nutrient availability in poultry feedstuffs. Anim. Feed Sci. Technol. 60:321- 330

Marquardt, R. R., 1997. Recommendations for future research on the use of enzymes in animal feeds, In: Enzymes in Poultry and Swine Nutrition. Ed. R.R. Marquardt and Z. Han, IDRC. pp.

May, R. W., and J. M. Bell, 1971. Digestible and metabolizable energy values of some feeds for the growing pig. Can. J. Anim. Sci. 51:271-278

Mellinkoff, S. M., M. Frankland, D. Boyle, and M. Geipell, 1956. Relationship between serum amino acid concentration and fluctuations in appetite. J. Appl. Physiol., 8: 535-538

Misir, R., and R. R. Marquardt, 1978. Factors affecting rye (Secale cerede L.) utilization growing chicks. I. The influence of rye level, ergot and penicillin supplementation. Can. J. Anim. Sci. 58:691-701

Mitaru, B. N., R. Blair, J. M. Bell, and R. D. Reichert, 1982. Tannin and fiber contents of rapeseed and canola hulls. Can. J. Anim. Sci. 62:661-663

Mitaru, B. N., R. Blair, J. M. Bell, and R. D. Reichert, 1983. Effect of canola hulls on growth, feed efficiency and protein and energy utilization in broiler chickens. Can. J. Anim. Sci. 63:655-662

Moore, S. 1963. On the determination of cysteine and cysteic acid. J. Biol. Chem 238:235-237

Namkung, H., and S. Leeson, 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poultry Sci. 78:1317-1319

National Research Council., 1994. Nutrient requirements of poultry. 9th revised edition. National Academy Press. Washington, D. C.

Nelson, T. S., 1967. The utilization of phytate phosphorus by poultry: a review. Poultry Sci. 46:862-871

Nelson, T. S., J. J. McGillivray, T. R. Shieh, R. J. Wodzinski, and J. H. Ware, 1968. Effect of phytate on the calcium requirement of chicks. Poultry Sci. 47:1985-1989

Nernberg, L. W. J., 1998. Improved phosphorus availability in poultry fed wheat/canola meal-based diets supplemented with phytase enzyme. M.Sc Thesis. University of Manitoba

Newkirk, R. W., H. L. Classen, and R. T. Tyler, 1997. Nutritional evaluation of low glucosinolate mustard meals (*Brassica juncea*) in broiler diets. Poultry Sci. 76:1272-1277

Newkirk, R. W., and H. L. Classen, 1998. *In vitro* hydrolysis of phytate in canola meal with purified and crude sources of phytase, Anim. Feed. Sci. Technol., 72:315-327

Noy, Y. and D. Sklan, 1995. Digestion and absorption in the young chicken. Poultry Science 74:366-373

Nutrition Guide, 1989. Rhone Poulenc Animal Nutiriton.

Nwokolo, E. N., and D. B. Bragg, 1977. Influence of phytic acid and crude fibre on the availability of minerals from four protein supplements in growing chicks. Can. J. Anim. Sci. 57:475-477

O'Dell, B. L., J. M. Yohe, and J. E. Savage, 1964. Zinc availability in the chick as affected by phytate, calcium, and ethylenediaminetetraacetate. Poultry Sci. 43: 415-419

Pettersson, D., H. Graham, and P. Aman, 1990. Enzyme supplementation of broiler chicken diets based on cereals with endosperm cell walls rich in arabinoxylans or mixed-linked β - glucan. Animal Production 51: 201-207

Puchal, F., and J. Mascarell, 1999. Role of feed enzymes in poultry nutrition examined. Feedstuffs. Nov.1:12-14

Ravindran, V., W. L. Bryden, and E. T. Kornegay, 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. Poultry and Avian Biology Reviews 6: 125-143

Ravindran, V., and W. L. Bryden, 1997. Influence of phytic acid and available phosphorus levels on the response of broilers to supplemental Natuphos. Poultry Res. Foundation Rep., University of Sidney, Australia.

Ravindran, V., P. H. Selle, and W. L. Bryden, 1999. Effect of phytase supplementation, individually and in combination, with glycanase on the nutritive of wheat and barley. Poultry Sci. 78:1585-1595

Rogers, Q. R. and P. M. B. Leung, 1973. The influence of amino acids on the neuroregulation of food intake. Fed. Proc. 32:1709

Rolls, B. A., J. W. G. Porter, and D. R. Westgarth, 1972. The course of digestion of different food proteins in the rat. 3. The absorption of proteins given alone and with supplements of their limiting amino acids. Br. J. Nutr. 28, 283-293

Rotter, B. A., R. R. Marquardt, W. Guenter, C. Biliaderis, and C. W. Newman, 1989. *In vitro* viscosity measurements of barley extracts as predictors of growth responses in chicks fed barley-based diets supplemented with a fungal enzyme preparation. Can. J. Anim. Sci. 69:431-439

Rotter, B. A., O. D. Friesen, W. Guenter, and R. R. Marquardt, 1990. Influence of enzyme supplementation on the bioavailable energy of barley. Poultry Sci. 69: 1174-1181

Salmon, W. P. 1954. The tryptophan requirement of the rat as affected by niacin and level of dietary nitrogen. Archives of Biochemistry and Biophysics. 51:30-41

Salmon, R. E., 1984a True metabolizable energy and dry matter contents of some feedstuffs. Poultry Sci. 63:381-383

Salmon, R. E., 1984b True metabolizable energy and total and available amino acids of candle, altex, and regent canola meals. Poultry Sci. 63:135-138

Sanahuja, J. C. and A. E. Harper, 1962. Effect of amino acid imbalance on food intake and preference. Amer. J. Physiol., 202:165-170

Sarwar, G., J. M. Bell, T. F. Sharby, and J. D. Jones, 1981. Nutritional evaluation of meals and meal fractions derived from rape and mustard seed. Can. J. Anim. Sci. 61:719-733

SAS Institute, 1990. SAS/STAT® User's Guide. Version 6, 4th ed. SAS Institute Inc., Carey, NC

Sauer, W. C., R. Cichon, and R. Misir, 1982. Amino acid availability and protein quality of canola and rapeseed meal for pigs and rats. J. Anim Sci.54:292-301

Sauer, W. C., and P. A. Thacker, 1986. Apparent ileal and faecal digestibility of amino acids in barley-based diets supplemented with soya bean meal or canola meal for growing pigs. Anim. Feed Sci. Technol. 14:183-192

Schöner, F. J., P. P. Hoppe, and G. Schwartz, 1991. Comparative effects of microbial phytase and inorganic phosphorus on performance and retentions of phosphorus, calcium and crude ash in broilers. J. of Anim. Physio. Anim. Nutr. 66:248-255

Sebastian, S., S. P. Touchburn, and E. R. Chavez, 1994. The effect of supplemental enzymes on the performence of broiler chicken fed different basal diets. McDonald College Report. McGill University, pp28-30

Sebastian, S., S. P. Touchbum, E. R. Chavez, and P. C. Lague, 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed a corn-soybean diets. Poultry Sci. 75:729-736

Sebastian, S., S. P. Touchburn, and E. R. Chavez, 1998. Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a review. World's Poultry. Science Journal 54:27-46

Serraino, M. R., L. U. Thompson, L. Savoie, and G. Parent, 1985. Effect of phytic acid on the *in vitro* rate of digestibility of rapeseed protein and amino acids. J. Food Sci. 50:1689-1692

Shires, A., J. M. Bell, W. H. Laverty, P. Fedec, J. A. Blake, and D. I. McGregor, 1983. Effect of desolventization conditions and removal of fibrous material by screening on the nutritional value of canola rapeseed meal for broiler chickens. Poultry Sci. 62:2234-2244

Shurlock, T. G. H. and J. M. Forbes, 1981. Factors affecting food intake in the domestic chicken: The effect of infusions of nutritive and non-nutritive substances into the crop and duodenum. British poultry science. 22:323-331

Siddiqui, I. R., and P. J. Wood, 1977. Carbohydrates of rapeseed: a review. J. Sci. Food Agric. 28: 530-538

Simbaya, J., B. A. Slominski, G. Rakow, L. D. Campbell, R. K. Downey, and J. M. Bell, 1995. Quality characteristics of yellow-Seeded brassica seed meals: protein, carbohydrates, and dietary fiber components. J. Agric. Food Chem. 43: 2062-2066

Simbaya, J., 1996. Potential for improved utilization of canola meal by monogastric animals. Ph. D. Thesis. University of Manitoba.

Simbaya, J., B. A. Slominski, W. Guenter, A. Morgan, and L. D. Campbell, 1996. The effects of protease and carbohydrase supplementation on the nutritive value of canola meal for poultry: *In vitro* and *in vivo* studies, Anim. Feed. Sci. Technol. 61:219-234

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. P. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor, 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540

Singh, M., and A. D. Krikorian, 1982. Inhibition of trypsin activity *in vitro* by phytate. J. Agric. Food Chem. 30:799-800

Slominski, B. A., and L. D. Campbell, 1990. Non-starch polysaccharides of canola meal: Quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 53:175-184

Slominski, B. A., and L. D. Campbell, 1991. The carbohydrate content of yellow-seeded canola. in: Proceedings of the 8th international rapeseed conference, saskatoon, SK, Canada. 1402-1407

Slominski, B. A., 1993. Canola Meal Quality Characteristics Proceedings to the Maryland Nutrition Conference. pp18-30

Slominski, B. A., L. D. Campbell, and W. Guenter, 1994. Oligosaccharides in canola meal and their effect on nonstarch polysaccharide digestibility and true metabolizable energy in poultry. Poultry Sci. 73:156-162

Slominski, B. A., 1994 Hydrolysis of galactooligosaccharides by commercial preparations of α-galactosidase and β-fructofuranosidase: Potential for use as dietary additives, J. Sci. Food. Agric., 65:323-330

Slominski, B. A., 1997. Developments in the breeding of low fibre rapeseed/canola. J. of Anim. Feed Sci. 6:303-317

Slominski, B. A., W. Guenter, and L. D. Campbell, 1997. Enzymes for canola meal: is blending of the existing products sufficient for development of an effective enzyme preparation? Poultry Sci. (Suppl.1) 76:79

Slominski, B. A., M. Cyran, W. Guenter, and L. D. Campbell, 1999. Nutritive value of enzyme-treated canola meal. Proceedings of the 10th international rapeseed congress, Sept. 26-29. 1999, Canberra, Australia.

Sosulski, K., F. Sosulski, and P. Thacker, 1990. Research on canola meal. An excerpt from the tenth project report. Canola Council of Canada. pp.44-63.

Temperton, H., and J. Cassidy, 1964. Phosphorus requirements of poultry. III. The effect of feeding a vegetable type diet without supplemental phosphorus to turkeys. Br. Poultry Sci. 5:87-88

Thompson, L. U., 1986. PA: a factor influencing starch digestibility and blood-glucose response, in Phytic Acid Chemistry and Application, ed. Graf, E., Minneapolis: Pilatus Press. 173-194

Tookey, H. L., C. H. Van Etten, and M. E. Daxenbichler, 1980. Glucosinolates. Ch. 4, pages 103-142, in Liener I. E., ed. Toxic Constituents of Plant Foodstuffs. 2th ed. Academic Press, New York, NY.

Vermorel, M., R. K. Heaney, and G. R. Fenwick, 1988. Antinutritional effects of the rapeseed meals, Darmor and Jet Neuf, and progoitrin together with myrosinase, in the growing rat. J. Sci. Food Agric.44: 321-334

White, W. B., R. H. Bird, M. L. Sunde, N. Prentice, W. C. Burger, and J. A. Marlett, 1981. The viscosity interaction of barley β-glucan *Trichoderma viride* cellulase in the chick intestine. Poultry Sci. 60:1043-1048

White, W. B., R. H. Bird, M. L. Sunde, and J. A. Marlett, Prentice, N. A. and Burger, W. C. 1983. Viscosity of β-glucan as a factor in the enzymatic improvement of barley for chicks. Poultry Sci. 62: 853-862

Wethli, E., T. R. Morris, and Schnesta, T. P. 1975. The effect of feeding high levels of low-quality proteins to growing chicks. Br. J. Nutr. 34: 363-378

Williams, C. H., D. J. David, and O. Lismoa, 1962. The determination of chromic oxide in fecal samples by atomic absorption spectrophotometry. J. Agric. Sci. 59:381-384

Yi, X., E. T. Kornegay, and D. M. Denbow, 1996. Effect of microbial phytase on nitrogen and amino acids digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. Poultry Sci. 75: 979-990

Zuprizal, M. L, and A. M. Chagneau, 1992. Effect of age and sex on true digestibility of amino acids of rapeseed and soybean meals in growing broilers. Poult. Sci. , 71: 1486-1492

Zyla, K. and J. Koreleski, 1993. *In vitro* and *in vivo* dephosphorylation of rapeseed meal by means of phytate-degrading enzymes derived from *Aspergillus niger,* J. Sci. Food Agric, 61:1-6